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(71) Applicant: CORIXA CORPORATION [US/US]; 1124 Columbia Street, Seattle, WA 98104 (US).

(72) Inventors: REED, Steven, G.; 2843 – 122nd Place N.E., Bellevue, WA 98005 (US). SKEIKY, Yasir, A., W.; 8327 – 25th Avenue N.W., Seattle, WA 98107 (US). DILLON, Davin, C.; 21607 N.E. 24th Street, Redmond, WA 98053 (US). CAMPOS–NETO, Antonio; 9308 Midship Court N.E., Bainbridge Island, WA 98021 (US). HOUGHTON, Raymond; 2636 – 242nd Place S.E., Bothell, WA 98021 (US). VEDVICK, Thomas, S.; 124 South 300th Place, Federal Way, WA 98003 (US). TWARDZIK, Daniel, R.; 10195 South Beach Drive, Bainbridge Island, WA 98110 (US). LODES, Michael, J.; 9223 – 36th Avenue S.W., Seattle, WA 98126 (US).

(74) Agents: MAKI, David, J. et al.; Seed and Berry LLP, 6300 Columbia Center, 701 Fifth Avenue, Seattle, WA 98104–7092 (US).

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(54) Title: COMPOUNDS AND METHODS FOR IMMUNOTHERAPY AND DIAGNOSIS OF TUBERCULOSIS

(57) Abstract

Compounds and methods for inducing protective immunity against tuberculosis are disclosed. The compounds provided include polypeptides that contain at least one immunogenic portion of one or more *M. tuberculosis* proteins and DNA molecules encoding such polypeptides. Such compounds may be formulated into vaccines and/or pharmaceutical compositions for immunization against *M. tuberculosis* infection, or may be used for the diagnosis of tuberculosis.

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COMPOUNDS AND METHODS FOR IMMUNOTHERAPY AND DIAGNOSIS OF TUBERCULOSIS

TECHNICAL FIELD

The present invention relates generally to detecting, treating and preventing *Mycobacterium tuberculosis* infection. The invention is more particularly related to polypeptides comprising a *Mycobacterium tuberculosis* antigen, or a portion or other variant thereof, and the use of such polypeptides for diagnosing and vaccinating against *Mycobacterium tuberculosis* infection.

BACKGROUND OF THE INVENTION

Tuberculosis is a chronic, infectious disease, that is generally caused by infection with *Mycobacterium tuberculosis*. It is a major disease in developing countries, as well as an increasing problem in developed areas of the world, with about 8 million new cases and 3 million deaths each year. Although the infection may be asymptomatic for a considerable period of time, the disease is most commonly manifested as an acute inflammation of the lungs, resulting in fever and a nonproductive cough. If left untreated, serious complications and death typically result.

Although tuberculosis can generally be controlled using extended antibiotic therapy, such treatment is not sufficient to prevent the spread of the disease. Infected individuals may be asymptomatic, but contagious, for some time. In addition, although compliance with the treatment regimen is critical, patient behavior is difficult to monitor. Some patients do not complete the course of treatment, which can lead to ineffective treatment and the development of drug resistance.

Inhibiting the spread of tuberculosis requires effective vaccination and accurate, early diagnosis of the disease. Currently, vaccination with live bacteria is the most efficient method for inducing protective immunity. The most common Mycobacterium employed for this purpose is *Bacillus* Calmette-Guerin (BCG), an avirulent strain of *Mycobacterium bovis*. However, the safety and efficacy of BCG is a source of controversy and some countries, such as the United States, do not vaccinate

the general public. Diagnosis is commonly achieved using a skin test, which involves intradermal exposure to tuberculin PPD (protein-purified derivative). Antigen-specific T cell responses result in measurable induration at the injection site by 48-72 hours after injection, which indicates exposure to Mycobacterial antigens. Sensitivity and specificity have, however, been a problem with this test, and individuals vaccinated with BCG cannot be distinguished from infected individuals.

While macrophages have been shown to act as the principal effectors of *M tuberculosis* immunity, T cells are the predominant inducers of such immunity. The essential role of T cells in protection against *M. tuberculosis* infection is illustrated by the frequent occurrence of *M. tuberculosis* in AIDS patients, due to the depletion of CD4 T cells associated with human immunodeficiency virus (HIV) infection. Mycobacterium-reactive CD4 T cells have been shown to be potent producers of gamma-interferon (IFN-γ), which, in turn, has been shown to trigger the anti-mycobacterial effects of macrophages in mice. While the role of IFN-γ in humans is less clear, studies have shown that 1,25-dihydroxy-vitamin D3, either alone or in combination with IFN-γ or tumor necrosis factor-alpha, activates human macrophages to inhibit *M. tuberculosis* infection. Furthermore, it is known that IFN-γ stimulates human macrophages to make 1,25-dihydroxy-vitamin D3. Similarly, IL-12 has been shown to play a role in stimulating resistance to *M. tuberculosis* infection. For a review of the immunology of *M. tuberculosis* infection see Chan and Kaufmann in *Tuberculosis: Pathogenesis, Protection and Control*, Bloom (ed.), ASM Press, Washington, DC, 1994.

Accordingly, there is a need in the art for improved vaccines and methods for preventing, treating and detecting tuberculosis. The present invention fulfills these needs and further provides other related advantages.

SUMMARY OF THE INVENTION

Briefly stated, this invention provides compounds and methods for preventing and diagnosing tuberculosis. In one aspect, polypeptides are provided comprising an immunogenic portion of a soluble *M. tuberculosis* antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. In

one embodiment of this aspect, the soluble antigen has one of the following N-terminal sequences:

(a) Asp-Pro-Val-Asp-Ala-Val-Ile-Asn-Thr-Thr-Cys-Asn-Tyr-Gly-Gln-Val-Val-Ala-Ala-Leu; (SEQ ID No. 120)

(b) Ala-Val-Glu-Ser-Gly-Met-Leu-Ala-Leu-Gly-Thr-Pro-Ala-Pro-Ser; (SEQ ID No. 121)

(c) Ala-Ala-Met-Lys-Pro-Arg-Thr-Gly-Asp-Gly-Pro-Leu-Glu-Ala-Ala-Lys-Glu-Gly-Arg; (SEQ ID No. 122)

(d) Tyr-Tyr-Trp-Cys-Pro-Gly-Gln-Pro-Phe-Asp-Pro-Ala-Trp-Gly-Pro; (SEQ ID No. 123)

(e) Asp-Ile-Gly-Ser-Glu-Ser-Thr-Glu-Asp-Gln-Gln-Xaa-Ala-Val; (SEQ ID No. 124)

(f) Ala-Glu-Glu-Ser-Ile-Ser-Thr-Xaa-Glu-Xaa-Ile-Val-Pro; (SEQ ID No. 125)

(g) Asp-Pro-Glu-Pro-Ala-Pro-Pro-Val-Pro-Thr-Thr-Ala-Ala-Ser-Pro-Pro-Ser; (SEQ ID No. 126)

(h) Ala-Pro-Lys-Thr-Tyr-Xaa-Glu-Glu-Leu-Lys-Gly-Thr-Asp-Thr-Gly; (SEQ ID No. 127)

(i) Asp-Pro-Ala-Ser-Ala-Pro-Asp-Val-Pro-Thr-Ala-Ala-Gln-Leu-Thr-Ser-Leu-Leu-Asn-Ser-Leu-Ala-Asp-Pro-Asn-Val-Ser-Phe-Ala-Asn; (SEQ ID No. 128)

(j) Xaa-Asp-Ser-Glu-Lys-Ser-Ala-Thr-Ile-Lys-Val-Thr-Asp-Ala-Ser; (SEQ ID No. 134)

(k) Ala-Gly-Asp-Thr-Xaa-Ile-Tyr-Ile-Val-Gly-Asn-Leu-Thr-Ala-Asp; (SEQ ID No. 135) or

(l) Ala-Pro-Glu-Ser-Gly-Ala-Gly-Leu-Gly-Gly-Thr-Val-Gln-Ala-Gly; (SEQ ID No. 136)

wherein Xaa may be any amino acid.

In a related aspect, polypeptides are provided comprising an immunogenic portion of an *M. tuberculosis* antigen, or a variant of such an antigen that

differs only in conservative substitutions and/or modifications, the antigen having one of the following N-terminal sequences:

(m) Xaa-Tyr-Ile-Ala-Tyr-Xaa-Thr-Thr-Ala-Gly-Ile-Val-Pro-Gly-Lys-Ile-Asn-Val-His-Leu-Val; (SEQ ID No. 137) or

(n) Asp-Pro-Pro-Asp-Pro-His-Gln-Xaa-Asp-Met-Thr-Lys-Gly-Tyr-Tyr-Pro-Gly-Gly-Arg-Arg-Xaa-Phe; (SEQ ID No. 129)

wherein Xaa may be any amino acid.

In another embodiment, the soluble *M. tuberculosis* antigen comprises an amino acid sequence encoded by a DNA sequence selected from the group consisting of the sequences recited in SEQ ID Nos.: 1, 2, 4-10, 13-25, 52, 99 and 101, the complements of said sequences, and DNA sequences that hybridize to a sequence recited in SEQ ID Nos.: 1, 2, 4-10, 13-25, 52, 99 and 101 or a complement thereof under moderately stringent conditions.

In a related aspect, the polypeptides comprise an immunogenic portion of a *M. tuberculosis* antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications, wherein the antigen comprises an amino acid sequence encoded by a DNA sequence selected from the group consisting of the sequences recited in SEQ ID Nos.: 26-51, 138, 139, 163-183 and 201, the complements of said sequences, and DNA sequences that hybridize to a sequence recited in SEQ ID Nos.: 26-51, 138, 139, 163-183 and 201 or a complement thereof under moderately stringent conditions.

In related aspects, DNA sequences encoding the above polypeptides, expression vectors comprising these DNA sequences and host cells transformed or transfected with such expression vectors are also provided.

In another aspect, the present invention provides fusion proteins comprising a first and a second inventive polypeptide or, alternatively, an inventive polypeptide and a known *M. tuberculosis* antigen.

Within other aspects, the present invention provides pharmaceutical compositions that comprise one or more of the above polypeptides, or a DNA molecule encoding such polypeptides, and a physiologically acceptable carrier. The invention

also provides vaccines comprising one or more of the polypeptides as described above and a non-specific immune response enhancer, together with vaccines comprising one or more DNA sequences encoding such polypeptides and a non-specific immune response enhancer.

In yet another aspect, methods are provided for inducing protective immunity in a patient, comprising administering to a patient an effective amount of one or more of the above polypeptides.

In further aspects of this invention, methods and diagnostic kits are provided for detecting tuberculosis in a patient. The methods comprise contacting dermal cells of a patient with one or more of the above polypeptides and detecting an immune response on the patient's skin. The diagnostic kits comprise one or more of the above polypeptides in combination with an apparatus sufficient to contact the polypeptide with the dermal cells of a patient.

In yet other aspects, methods are provided for detecting tuberculosis in a patient, such methods comprising contacting dermal cells of a patient with one or more polypeptides encoded by a DNA sequence selected from the group consisting of SEQ ID Nos.: 3, 11, 12, 140, 141, 156-160, 189-193, 199, 200 and 203, the complements of said sequences, and DNA sequences that hybridize to a sequence recited in SEQ ID Nos.: 3, 11, 12, 140, 141, 156-160, 189-193, 199, 200 and 203; and detecting an immune response on the patient's skin. Diagnostic kits for use in such methods are also provided.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCE IDENTIFIERS

Figure 1A and B illustrate the stimulation of proliferation and interferon-γ production in T cells derived from a first and a second *M. tuberculosis*-immune donor, respectively, by the 14 Kd, 20 Kd and 26 Kd antigens described in Example 1.

Figure 2 illustrates the stimulation of proliferation and interferon-γ production in T cells derived from an *M. tuberculosis*-immune individual by the two representative polypeptides TbRa3 and TbRa9.

Figures 3A-D illustrate the reactivity of antisera raised against secretory *M. tuberculosis* proteins, the known *M. tuberculosis* antigen 85b and the inventive antigens Tb38-1 and TbH-9, respectively, with *M. tuberculosis* lysate (lane 2), *M. tuberculosis* secretory proteins (lane 3), recombinant Tb38-1 (lane 4), recombinant TbH-9 (lane 5) and recombinant 85b (lane 5).

Figure 4A illustrates the stimulation of proliferation in a TbH-9-specific T cell clone by secretory *M. tuberculosis* proteins, recombinant TbH-9 and a control antigen, TbRa11.

Figure 4B illustrates the stimulation of interferon-γ production in a TbH-9-specific T cell clone by secretory *M. tuberculosis* proteins, PPD and recombinant TbH-9.

Figures 5A and B illustrate the stimulation of proliferation and interferon-γ production in TbH9-specific T cells by the fusion protein TbH9-Tb38-1.

Figures 6A and B illustrate the stimulation of proliferation and interferon-γ production in Tb38-1-specific T cells by the fusion protein TbH9-Tb38-1.

Figures 7A and B illustrate the stimulation of proliferation and interferon-γ production in T cells previously shown to respond to both TbH-9 and Tb38-1 by the fusion protein TbH9-Tb38-1.

Figures 8A and B illustrate the stimulation of proliferation and interferon-γ production in T cells derived from a first *M. tuberculosis*-immune individual by the representative polypeptides XP-1, RDIF6, RDIF8, RDIF10 and RDIF11.

Figures 9A and B illustrate the stimulation of proliferation and interferon-γ production in T cells derived from a second *M. tuberculosis*-immune individual by the representative polypeptides XP-1, RDIF6, RDIF8, RDIF10 and RDIF11.

SEQ. ID NO. 1 is the DNA sequence of TbRa1.

SEQ. ID NO. 2 is the DNA sequence of TbRa10.

SEQ. ID NO. 3 is the DNA sequence of TbRa11.

SEQ. ID NO. 4 is the DNA sequence of TbRa12.

SEQ. ID NO. 5 is the DNA sequence of TbRa13.

SEQ. ID NO. 6 is the DNA sequence of TbRa16.

SEQ. ID NO. 7 is the DNA sequence of TbRa17.

SEQ. ID NO. 8 is the DNA sequence of TbRa18.

SEQ. ID NO. 9 is the DNA sequence of TbRa19.

SEQ. ID NO. 10 is the DNA sequence of TbRa24.

SEQ. ID NO. 11 is the DNA sequence of TbRa26.

SEQ. ID NO. 12 is the DNA sequence of TbRa28.

SEQ. ID NO. 13 is the DNA sequence of TbRa29.

SEQ. ID NO. 14 is the DNA sequence of TbRa2A.

SEQ. ID NO. 15 is the DNA sequence of TbRa3.

SEQ. ID NO. 16 is the DNA sequence of TbRa32.

SEQ. ID NO. 17 is the DNA sequence of TbRa35.

SEQ. ID NO. 18 is the DNA sequence of TbRa36.

SEQ. ID NO. 19 is the DNA sequence of TbRa4.

SEQ. ID NO. 20 is the DNA sequence of TbRa9.

SEQ. ID NO. 21 is the DNA sequence of TbRaB.

SEQ. ID NO. 22 is the DNA sequence of TbRaC.

SEQ. ID NO. 23 is the DNA sequence of TbRaD.

SEQ. ID NO. 24 is the DNA sequence of YYWCPG.

SEQ. ID NO. 25 is the DNA sequence of AAMK.

SEQ. ID NO. 26 is the DNA sequence of TbL-23.

SEQ. ID NO. 27 is the DNA sequence of TbL-24.

SEQ. ID NO. 28 is the DNA sequence of TbL-25.

SEQ. ID NO. 29 is the DNA sequence of TbL-28.

SEQ. ID NO. 30 is the DNA sequence of TbL-29.

SEQ. ID NO. 31 is the DNA sequence of TbH-5.

SEQ. ID NO. 32 is the DNA sequence of TbH-8.

SEQ. ID NO. 33 is the DNA sequence of TbH-9.

SEQ. ID NO. 34 is the DNA sequence of TbM-1.

SEQ. ID NO. 35 is the DNA sequence of TbM-3.

SEQ. ID NO. 36 is the DNA sequence of TbM-6.

SEQ. ID NO. 37 is the DNA sequence of TbM-7.

SEQ. ID NO. 38 is the DNA sequence of TbM-9.

SEQ. ID NO. 39 is the DNA sequence of TbM-12.

SEQ. ID NO. 40 is the DNA sequence of TbM-13.

SEQ. ID NO. 41 is the DNA sequence of TbM-14.

SEQ. ID NO. 42 is the DNA sequence of TbM-15.

SEQ. ID NO. 43 is the DNA sequence of TbH-4.

SEQ. ID NO. 44 is the DNA sequence of TbH-4-FWD.

SEQ. ID NO. 45 is the DNA sequence of TbH-12.

SEQ. ID NO. 46 is the DNA sequence of Tb38-1.

SEQ. ID NO. 47 is the DNA sequence of Tb38-4.

SEQ. ID NO. 48 is the DNA sequence of TbL-17.

SEQ. ID NO. 49 is the DNA sequence of TbL-20.

SEQ. ID NO. 50 is the DNA sequence of TbL-21.

SEQ. ID NO. 51 is the DNA sequence of TbH-16.

SEQ. ID NO. 52 is the DNA sequence of DPEP.

SEQ. ID NO. 53 is the deduced amino acid sequence of DPEP.

SEQ. ID NO. 54 is the protein sequence of DPV N-terminal Antigen.

SEQ. ID NO. 55 is the protein sequence of AVGS N-terminal Antigen.

SEQ. ID NO. 56 is the protein sequence of AAMK N-terminal Antigen.

SEQ. ID NO. 57 is the protein sequence of YYWC N-terminal Antigen.

SEQ. ID NO. 58 is the protein sequence of DIGS N-terminal Antigen.

SEQ. ID NO. 59 is the protein sequence of AEES N-terminal Antigen.

SEQ. ID NO. 60 is the protein sequence of DPEP N-terminal Antigen.

SEQ. ID NO. 61 is the protein sequence of APKT N-terminal Antigen.

SEQ. ID NO. 62 is the protein sequence of DPAS N-terminal Antigen.

SEQ. ID NO. 63 is the deduced amino acid sequence of TbRa1.

SEQ. ID NO. 64 is the deduced amino acid sequence of TbRa10.

SEQ. ID NO. 65 is the deduced amino acid sequence of TbRa11.

SEQ. ID NO. 66 is the deduced amino acid sequence of TbRa12.

SEQ. ID NO. 67 is the deduced amino acid sequence of TbRa13.

SEQ. ID NO. 68 is the deduced amino acid sequence of TbRa16.

SEQ. ID NO. 69 is the deduced amino acid sequence of TbRa17.

SEQ. ID NO. 70 is the deduced amino acid sequence of TbRa18.

SEQ. ID NO. 71 is the deduced amino acid sequence of TbRa19.

SEQ. ID NO. 72 is the deduced amino acid sequence of TbRa24.

SEQ. ID NO. 73 is the deduced amino acid sequence of TbRa26.

SEQ. ID NO. 74 is the deduced amino acid sequence of TbRa28.

SEQ. ID NO. 75 is the deduced amino acid sequence of TbRa29.

SEQ. ID NO. 76 is the deduced amino acid sequence of TbRa2A.

SEQ. ID NO. 77 is the deduced amino acid sequence of TbRa3.

SEQ. ID NO. 78 is the deduced amino acid sequence of TbRa32.

SEQ. ID NO. 79 is the deduced amino acid sequence of TbRa35.

SEQ. ID NO. 80 is the deduced amino acid sequence of TbRa36.

SEQ. ID NO. 81 is the deduced amino acid sequence of TbRa4.

SEQ. ID NO. 82 is the deduced amino acid sequence of TbRa9.

SEQ. ID NO. 83 is the deduced amino acid sequence of TbRaB.

SEQ. ID NO. 84 is the deduced amino acid sequence of TbRaC.

SEQ. ID NO. 85 is the deduced amino acid sequence of TbRaD.

SEQ. ID NO. 86 is the deduced amino acid sequence of YYWCPG.

SEQ. ID NO. 87 is the deduced amino acid sequence of TbAAMK.

SEQ. ID NO. 88 is the deduced amino acid sequence of Tb38-1.

SEQ. ID NO. 89 is the deduced amino acid sequence of TbH-4.

SEQ. ID NO. 90 is the deduced amino acid sequence of TbH-8.

SEQ. ID NO. 91 is the deduced amino acid sequence of TbH-9.

SEQ. ID NO. 92 is the deduced amino acid sequence of TbH-12.

SEQ. ID NO. 93 is the amino acid sequence of Tb38-1 Peptide 1.

SEQ. ID NO. 94 is the amino acid sequence of Tb38-1 Peptide 2.

SEQ. ID NO. 95 is the amino acid sequence of Tb38-1 Peptide 3.

SEQ. ID NO. 96 is the amino acid sequence of Tb38-1 Peptide 4.

SEQ. ID NO. 97 is the amino acid sequence of Tb38-1 Peptide 5.

SEQ. ID NO. 98 is the amino acid sequence of Tb38-1 Peptide 6.

SEQ. ID NO. 99 is the DNA sequence of DPAS.

SEQ. ID NO. 100 is the deduced amino acid sequence of DPAS.

SEQ. ID NO. 101 is the DNA sequence of DPV.

SEQ. ID NO. 102 is the deduced amino acid sequence of DPV.

SEQ. ID NO. 103 is the DNA sequence of ESAT-6.

SEQ. ID NO. 104 is the deduced amino acid sequence of ESAT-6.

SEQ. ID NO. 105 is the DNA sequence of TbH-8-2.

SEQ. ID NO. 106 is the DNA sequence of TbH-9FL.

SEQ. ID NO. 107 is the deduced amino acid sequence of TbH-9FL.

SEQ. ID NO. 108 is the DNA sequence of TbH-9-1.

SEQ. ID NO. 109 is the deduced amino acid sequence of TbH-9-1.

SEQ. ID NO. 110 is the DNA sequence of TbH-9-4.

SEQ. ID NO. 111 is the deduced amino acid sequence of TbH-9-4.

SEQ. ID NO. 112 is the DNA sequence of Tb38-1F2 IN.

SEQ. ID NO. 113 is the DNA sequence of Tb38-2F2 RP.

SEQ. ID NO. 114 is the deduced amino acid sequence of Tb37-FL.

SEQ. ID NO. 115 is the deduced amino acid sequence of Tb38-IN.

SEQ. ID NO. 116 is the DNA sequence of Tb38-1F3.

SEQ. ID NO. 117 is the deduced amino acid sequence of Tb38-1F3.

SEQ. ID NO. 118 is the DNA sequence of Tb38-1F5.

SEQ. ID NO. 119 is the DNA sequence of Tb38-1F6.

SEQ. ID NO. 120 is the deduced N-terminal amino acid sequence of DPV.

SEQ. ID NO. 121 is the deduced N-terminal amino acid sequence of AVGS.

SEQ. ID NO. 122 is the deduced N-terminal amino acid sequence of AAMK.

SEQ. ID NO. 123 is the deduced N-terminal amino acid sequence of YYWC.

SEQ. ID NO. 124 is the deduced N-terminal amino acid sequence of DIGS.

SEQ. ID NO. 125 is the deduced N-terminal amino acid sequence of AEES.

SEQ. ID NO. 126 is the deduced N-terminal amino acid sequence of DPEP.

SEQ. ID NO. 127 is the deduced N-terminal amino acid sequence of APKT.

SEQ. ID NO. 128 is the deduced amino acid sequence of DPAS.

SEQ. ID NO. 129 is the protein sequence of DPPD N-terminal Antigen.

SEQ ID NO. 130-133 are the protein sequences of four DPPD cyanogen bromide fragments.

SEQ ID NO. 134 is the N-terminal protein sequence of XDS antigen.

SEQ ID NO. 135 is the N-terminal protein sequence of AGD antigen.

SEQ ID NO. 136 is the N-terminal protein sequence of APE antigen.

SEQ ID NO. 137 is the N-terminal protein sequence of XYI antigen.

SEQ ID NO. 138 is the DNA sequence of TbH-29.

SEQ ID NO. 139 is the DNA sequence of TbH-30.

SEQ ID NO. 140 is the DNA sequence of TbH-32.

SEQ ID NO. 141 is the DNA sequence of TbH-33.

SEQ ID NO. 142 is the predicted amino acid sequence of TbH-29.

SEQ ID NO. 143 is the predicted amino acid sequence of TbH-30.

SEQ ID NO. 144 is the predicted amino acid sequence of TbH-32.

SEQ ID NO. 145 is the predicted amino acid sequence of TbH-33.

SEQ ID NO: 146-151 are PCR primers used in the preparation of a fusion protein containing TbRa3, 38 kD and Tb38-1.

SEQ ID NO: 152 is the DNA sequence of the fusion protein containing TbRa3, 38 kD and Tb38-1.

SEQ ID NO: 153 is the amino acid sequence of the fusion protein containing TbRa3, 38 kD and Tb38-1.

SEQ ID NO: 154 is the DNA sequence of the *M. tuberculosis* antigen 38 kD.

SEQ ID NO: 155 is the amino acid sequence of the *M. tuberculosis* antigen 38 kD.

SEQ ID NO: 156 is the DNA sequence of XP14.

SEQ ID NO: 157 is the DNA sequence of XP24.

SEQ ID NO: 158 is the DNA sequence of XP31.

SEQ ID NO: 159 is the 5' DNA sequence of XP32.

SEQ ID NO: 160 is the 3' DNA sequence of XP32.

SEQ ID NO: 161 is the predicted amino acid sequence of XP14.

SEQ ID NO: 162 is the predicted amino acid sequence encoded by the reverse complement of XP14.

SEQ ID NO: 163 is the DNA sequence of XP27.

SEQ ID NO: 164 is the DNA sequence of XP36.

SEQ ID NO: 165 is the 5' DNA sequence of XP4.

SEQ ID NO: 166 is the 5' DNA sequence of XP5.

SEQ ID NO: 167 is the 5' DNA sequence of XP17.

SEQ ID NO: 168 is the 5' DNA sequence of XP30.

SEQ ID NO: 169 is the 5' DNA sequence of XP2.

SEQ ID NO: 170 is the 3' DNA sequence of XP2.

SEQ ID NO: 171 is the 5' DNA sequence of XP3.

SEQ ID NO: 172 is the 3' DNA sequence of XP3.

SEQ ID NO: 173 is the 5' DNA sequence of XP6.

SEQ ID NO: 174 is the 3' DNA sequence of XP6.

SEQ ID NO: 175 is the 5' DNA sequence of XP18.

SEQ ID NO: 176 is the 3' DNA sequence of XP18.

SEQ ID NO: 177 is the 5' DNA sequence of XP19.

SEQ ID NO: 178 is the 3' DNA sequence of XP19.

SEQ ID NO: 179 is the 5' DNA sequence of XP22.

SEQ ID NO: 180 is the 3' DNA sequence of XP22.

SEQ ID NO: 181 is the 5' DNA sequence of XP25.

SEQ ID NO: 182 is the 3' DNA sequence of XP25.

SEQ ID NO: 183 is the full-length DNA sequence of TbH4-XP1.

SEQ ID NO: 184 is the predicted amino acid sequence of TbH4-XP1.

SEQ ID NO: 185 is the predicted amino acid sequence encoded by the reverse complement of TbH4-XP1.

SEQ ID NO: 186 is a first predicted amino acid sequence encoded by XP36.

SEQ ID NO: 187 is a second predicted amino acid sequence encoded by XP36.

SEQ ID NO: 188 is the predicted amino acid sequence encoded by the reverse complement of XP36.

SEQ ID NO: 189 is the DNA sequence of RDIF2.

SEQ ID NO: 190 is the DNA sequence of RDIF5.

SEQ ID NO: 191 is the DNA sequence of RDIF8.

SEQ ID NO: 192 is the DNA sequence of RDIF10.

SEQ ID NO: 193 is the DNA sequence of RDIF11.

SEQ ID NO: 194 is the predicted amino acid sequence of RDIF2.

SEQ ID NO: 195 is the predicted amino acid sequence of RDIF5.

SEQ ID NO: 196 is the predicted amino acid sequence of RDIF8.

SEQ ID NO: 197 is the predicted amino acid sequence of RDIF10.

SEQ ID NO: 198 is the predicted amino acid sequence of RDIF11.

SEQ ID NO: 199 is the 5' DNA sequence of RDIF12.

SEQ ID NO: 200 is the 3' DNA sequence of RDIF12.

SEQ ID NO: 201 is the DNA sequence of RDIF7.

SEQ ID NO: 202 is the predicted amino acid sequence of RDIF7.

SEQ ID NO: 203 is the DNA sequence of DIF2-1.

SEQ ID NO: 204 is the predicted amino acid sequence of DIF2-1.

SEQ ID NO: 205-212 are PCR primers used in the preparation of a fusion protein containing TbRa3, 38 kD, Tb38-1 and DPEP (hereinafter referred to as TbF-2).

SEQ ID NO: 213 is the DNA sequence of the fusion protein TbF-2.

SEQ ID NO: 214 is the amino acid sequence of the fusion protein TbF-2.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compositions and methods for preventing, treating and diagnosing tuberculosis. The compositions of the subject invention include polypeptides that comprise at least one immunogenic portion of a *M. tuberculosis* antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. Polypeptides within the scope of the present invention include, but are not limited to, immunogenic soluble *M. tuberculosis* antigens. A "soluble *M. tuberculosis* antigen" is a protein of *M. tuberculosis* origin that is present in *M. tuberculosis* culture filtrate. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins (*i.e.*, antigens), wherein the amino acid residues are linked by covalent peptide bonds. Thus, a polypeptide comprising an immunogenic portion of one of the above antigens may consist entirely of the immunogenic portion, or may contain additional sequences. The additional sequences may be derived from the native *M. tuberculosis* antigen or may be heterologous, and such sequences may (but need not) be immunogenic.

"Immunogenic," as used herein, refers to the ability to elicit an immune response (*e.g.*, cellular) in a patient, such as a human, and/or in a biological sample. In particular, antigens that are immunogenic (and immunogenic portions or other variants of such antigens) are capable of stimulating cell proliferation, interleukin-12 production and/or interferon-γ production in biological samples comprising one or more cells selected from the group of T cells, NK cells, B cells and macrophages, where the cells are derived from an *M. tuberculosis*-immune individual. Polypeptides comprising at least an immunogenic portion of one or more *M. tuberculosis* antigens may generally be used to detect tuberculosis or to induce protective immunity against tuberculosis in a patient.

The compositions and methods of this invention also encompass variants of the above polypeptides. A "variant," as used herein, is a polypeptide that differs from the native antigen only in conservative substitutions and/or modifications, such that the ability of the polypeptide to induce an immune response is retained. Such variants may generally be identified by modifying one of the above polypeptide

sequences, and evaluating the immunogenic properties of the modified polypeptide using, for example, the representative procedures described herein.

A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. In general, the following groups of amino acids represent conservative changes: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenic properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (*e.g.*, poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

In a related aspect, combination polypeptides are disclosed. A "combination polypeptide" is a polypeptide comprising at least one of the above immunogenic portions and one or more additional immunogenic *M. tuberculosis* sequences, which are joined via a peptide linkage into a single amino acid chain. The sequences may be joined directly (*i.e.*, with no intervening amino acids) or may be joined by way of a linker sequence (*e.g.*, Gly-Cys-Gly) that does not significantly diminish the immunogenic properties of the component polypeptides.

In general, *M. tuberculosis* antigens, and DNA sequences encoding such antigens, may be prepared using any of a variety of procedures. For example, soluble antigens may be isolated from *M. tuberculosis* culture filtrate by procedures known to those of ordinary skill in the art, including anion-exchange and reverse phase chromatography. Purified antigens are then evaluated for their ability to elicit an

appropriate immune response (*e.g.*, cellular) using, for example, the representative methods described herein. Immunogenic antigens may then be partially sequenced using techniques such as traditional Edman chemistry. *See* Edman and Berg, *Eur. J. Biochem. 80*:116-132, 1967.

Immunogenic antigens may also be produced recombinantly using a DNA sequence that encodes the antigen, which has been inserted into an expression vector and expressed in an appropriate host. DNA molecules encoding soluble antigens may be isolated by screening an appropriate *M. tuberculosis* expression library with anti-sera (*e.g.*, rabbit) raised specifically against soluble *M. tuberculosis* antigens. DNA sequences encoding antigens that may or may not be soluble may be identified by screening an appropriate *M. tuberculosis* genomic or cDNA expression library with sera obtained from patients infected with *M. tuberculosis*. Such screens may generally be performed using techniques well known to those of ordinary skill in the art, such as those described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989.

DNA sequences encoding soluble antigens may also be obtained by screening an appropriate *M. tuberculosis* cDNA or genomic DNA library for DNA sequences that hybridize to degenerate oligonucleotides derived from partial amino acid sequences of isolated soluble antigens. Degenerate oligonucleotide sequences for use in such a screen may be designed and synthesized, and the screen may be performed, as described (for example) in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989 (and references cited therein). Polymerase chain reaction (PCR) may also be employed, using the above oligonucleotides in methods well known in the art, to isolate a nucleic acid probe from a cDNA or genomic library. The library screen may then be performed using the isolated probe.

Alternatively, genomic or cDNA libraries derived from *M. tuberculosis* may be screened directly using peripheral blood mononuclear cells (PBMCs) or T cell lines or clones derived from one or more *M. tuberculosis*-immune individuals. In general, PBMCs and/or T cells for use in such screens may be prepared as described

below. Direct library screens may generally be performed by assaying pools of expressed recombinant proteins for the ability to induce proliferation and/or interferon-γ production in T cells derived from an *M. tuberculosis*-immune individual. Alternatively, potential T cell antigens may be first selected based on antibody reactivity, as described above.

Regardless of the method of preparation, the antigens (and immunogenic portions thereof) described herein (which may or may not be soluble) have the ability to induce an immunogenic response. More specifically, the antigens have the ability to induce proliferation and/or cytokine production (*i.e.*, interferon-γ and/or interleukin-12 production) in T cells, NK cells, B cells and/or macrophages derived from an *M. tuberculosis*-immune individual. The selection of cell type for use in evaluating an immunogenic response to a antigen will, of course, depend on the desired response. For example, interleukin-12 production is most readily evaluated using preparations containing B cells and/or macrophages. An *M. tuberculosis*-immune individual is one who is considered to be resistant to the development of tuberculosis by virtue of having mounted an effective T cell response to *M. tuberculosis* (*i.e.*, substantially free of disease symptoms). Such individuals may be identified based on a strongly positive (*i.e.*, greater than about 10 mm diameter induration) intradermal skin test response to tuberculosis proteins (PPD) and an absence of any signs or symptoms of tuberculosis disease. T cells, NK cells, B cells and macrophages derived from *M. tuberculosis*-immune individuals may be prepared using methods known to those of ordinary skill in the art. For example, a preparation of PBMCs (*i.e.*, peripheral blood mononuclear cells) may be employed without further separation of component cells. PBMCs may generally be prepared, for example, using density centrifugation through Ficoll™ (Winthrop Laboratories, NY). T cells for use in the assays described herein may also be purified directly from PBMCs. Alternatively, an enriched T cell line reactive against mycobacterial proteins, or T cell clones reactive to individual mycobacterial proteins, may be employed. Such T cell clones may be generated by, for example, culturing PBMCs from *M. tuberculosis*-immune individuals with mycobacterial proteins for a period of 2-4 weeks. This allows expansion of only the mycobacterial protein-specific

T cells, resulting in a line composed solely of such cells. These cells may then be cloned and tested with individual proteins, using methods known to those of ordinary skill in the art, to more accurately define individual T cell specificity. In general, antigens that test positive in assays for proliferation and/or cytokine production (*i.e.*, interferon-γ and/or interleukin-12 production) performed using T cells, NK cells, B cells and/or macrophages derived from an *M. tuberculosis*-immune individual are considered immunogenic. Such assays may be performed, for example, using the representative procedures described below. Immunogenic portions of such antigens may be identified using similar assays, and may be present within the polypeptides described herein.

The ability of a polypeptide (*e.g.*, an immunogenic antigen, or a portion or other variant thereof) to induce cell proliferation is evaluated by contacting the cells (*e.g.*, T cells and/or NK cells) with the polypeptide and measuring the proliferation of the cells. In general, the amount of polypeptide that is sufficient for evaluation of about 10^5 cells ranges from about 10 ng/mL to about 100 μg/mL and preferably is about 10 μg/mL. The incubation of polypeptide with cells is typically performed at 37°C for about six days. Following incubation with polypeptide, the cells are assayed for a proliferative response, which may be evaluated by methods known to those of ordinary skill in the art, such as exposing cells to a pulse of radiolabeled thymidine and measuring the incorporation of label into cellular DNA. In general, a polypeptide that results in at least a three fold increase in proliferation above background (*i.e.*, the proliferation observed for cells cultured without polypeptide) is considered to be able to induce proliferation.

The ability of a polypeptide to stimulate the production of interferon-γ and/or interleukin-12 in cells may be evaluated by contacting the cells with the polypeptide and measuring the level of interferon-γ or interleukin-12 produced by the cells. In general, the amount of polypeptide that is sufficient for the evaluation of about 10^5 cells ranges from about 10 ng/mL to about 100 μg/mL and preferably is about 10 μg/mL. The polypeptide may, but need not, be immobilized on a solid support, such as a bead or a biodegradable microsphere, such as those described in U.S. Patent Nos. 4,897,268 and 5,075,109. The incubation of polypeptide with the cells is typically

performed at 37°C for about six days. Following incubation with polypeptide, the cells are assayed for interferon-γ and/or interleukin-12 (or one or more subunits thereof), which may be evaluated by methods known to those of ordinary skill in the art, such as an enzyme-linked immunosorbent assay (ELISA) or, in the case of IL-12 P70 subunit, a bioassay such as an assay measuring proliferation of T cells. In general, a polypeptide that results in the production of at least 50 pg of interferon-γ per mL of cultured supernatant (containing 10^4-10^5 T cells per mL) is considered able to stimulate the production of interferon-γ. A polypeptide that stimulates the production of at least 10 pg/mL of IL-12 P70 subunit, and/or at least 100 pg/mL of IL-12 P40 subunit, per 10^5 macrophages or B cells (or per 3 x 10^5 PBMC) is considered able to stimulate the production of IL-12.

In general, immunogenic antigens are those antigens that stimulate proliferation and/or cytokine production (*i.e.*, interferon-γ and/or interleukin-12 production) in T cells, NK cells, B cells and/or macrophages derived from at least about 25% of *M. tuberculosis*-immune individuals. Among these immunogenic antigens, polypeptides having superior therapeutic properties may be distinguished based on the magnitude of the responses in the above assays and based on the percentage of individuals for which a response is observed. In addition, antigens having superior therapeutic properties will not stimulate proliferation and/or cytokine production *in vitro* in cells derived from more than about 25% of individuals that are not *M. tuberculosis*-immune, thereby eliminating responses that are not specifically due to *M. tuberculosis*-responsive cells. Those antigens that induce a response in a high percentage of T cell, NK cell, B cell and/or macrophage preparations from *M. tuberculosis*-immune individuals (with a low incidence of responses in cell preparations from other individuals) have superior therapeutic properties.

Antigens with superior therapeutic properties may also be identified based on their ability to diminish the severity of *M. tuberculosis* infection in experimental animals, when administered as a vaccine. Suitable vaccine preparations for use on experimental animals are described in detail below. Efficacy may be determined based on the ability of the antigen to provide at least about a 50% reduction

in bacterial numbers and/or at least about a 40% decrease in mortality following experimental infection. Suitable experimental animals include mice, guinea pigs and primates.

Antigens having superior diagnostic properties may generally be identified based on the ability to elicit a response in an intradermal skin test performed on an individual with active tuberculosis, but not in a test performed on an individual who is not infected with *M. tuberculosis*. Skin tests may generally be performed as described below, with a response of at least 5 mm induration considered positive.

Immunogenic portions of the antigens described herein may be prepared and identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3d ed., Raven Press, 1993, pp. 243-247 and references cited therein. Such techniques include screening polypeptide portions of the native antigen for immunogenic properties. The representative proliferation and cytokine production assays described herein may generally be employed in these screens. An immunogenic portion of a polypeptide is a portion that, within such representative assays, generates an immune response (*e.g.*, proliferation, interferon-γ production and/or interleukin-12 production) that is substantially similar to that generated by the full length antigen. In other words, an immunogenic portion of an antigen may generate at least about 20%, and preferably about 100%, of the proliferation induced by the full length antigen in the model proliferation assay described herein. An immunogenic portion may also, or alternatively, stimulate the production of at least about 20%, and preferably about 100%, of the interferon-γ and/or interleukin-12 induced by the full length antigen in the model assay described herein.

Portions and other variants of *M. tuberculosis* antigens may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See* Merrifield, *J. Am. Chem. Soc.*

85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Applied BioSystems, Inc., Foster City, CA, and may be operated according to the manufacturer's instructions. Variants of a native antigen may generally be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis. Sections of the DNA sequence may also be removed using standard techniques to permit preparation of truncated polypeptides.

Recombinant polypeptides containing portions and/or variants of a native antigen may be readily prepared from a DNA sequence encoding the polypeptide using a variety of techniques well known to those of ordinary skill in the art. For example, supernatants from suitable host/vector systems which secrete recombinant protein into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant protein.

Any of a variety of expression vectors known to those of ordinary skill in the art may be employed to express recombinant polypeptides of this invention. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line such as COS or CHO. The DNA sequences expressed in this manner may encode naturally occurring antigens, portions of naturally occurring antigens, or other variants thereof.

In general, regardless of the method of preparation, the polypeptides disclosed herein are prepared in substantially pure form. Preferably, the polypeptides are at least about 80% pure, more preferably at least about 90% pure and most preferably at least about 99% pure. In certain preferred embodiments, described in

detail below, the substantially pure polypeptides are incorporated into pharmaceutical compositions or vaccines for use in one or more of the methods disclosed herein.

In certain specific embodiments, the subject invention discloses polypeptides comprising at least an immunogenic portion of a soluble *M. tuberculosis* antigen having one of the following N-terminal sequences, or a variant thereof that differs only in conservative substitutions and/or modifications:

(a) Asp-Pro-Val-Asp-Ala-Val-Ile-Asn-Thr-Thr-Cys-Asn-Tyr-Gly-Gln-Val-Val-Ala-Ala-Leu; (SEQ ID No. 120)

(b) Ala-Val-Glu-Ser-Gly-Met-Leu-Ala-Leu-Gly-Thr-Pro-Ala-Pro-Ser; (SEQ ID No. 121)

(c) Ala-Ala-Met-Lys-Pro-Arg-Thr-Gly-Asp-Gly-Pro-Leu-Glu-Ala-Ala-Lys-Glu-Gly-Arg; (SEQ ID No. 122)

(d) Tyr-Tyr-Trp-Cys-Pro-Gly-Gln-Pro-Phe-Asp-Pro-Ala-Trp-Gly-Pro; (SEQ ID No. 123)

(e) Asp-Ile-Gly-Ser-Glu-Ser-Thr-Glu-Asp-Gln-Gln-Xaa-Ala-Val; (SEQ ID No. 124)

(f) Ala-Glu-Glu-Ser-Ile-Ser-Thr-Xaa-Glu-Xaa-Ile-Val-Pro; (SEQ ID No. 125)

(g) Asp-Pro-Glu-Pro-Ala-Pro-Pro-Val-Pro-Thr-Ala-Ala-Ala-Ser-Pro-Pro-Ser; (SEQ ID No. 126)

(h) Ala-Pro-Lys-Thr-Tyr-Xaa-Glu-Glu-Leu-Lys-Gly-Thr-Asp-Thr-Gly; (SEQ ID No. 127)

(i) Asp-Pro-Ala-Ser-Ala-Pro-Asp-Val-Pro-Thr-Ala-Ala-Gln-Leu-Thr-Ser-Leu-Leu-Asn-Ser-Leu-Ala-Asp-Pro-Asn-Val-Ser-Phe-Ala-Asn; (SEQ ID No. 128)

(j) Xaa-Asp-Ser-Glu-Lys-Ser-Ala-Thr-Ile-Lys-Val-Thr-Asp-Ala-Ser; (SEQ ID No. 134)

(k) Ala-Gly-Asp-Thr-Xaa-Ile-Tyr-Ile-Val-Gly-Asn-Leu-Thr-Ala-Asp; (SEQ ID No. 135) or

(l) Ala-Pro-Glu-Ser-Gly-Ala-Gly-Leu-Gly-Gly-Thr-Val-Gln-Ala-Gly; (SEQ ID No. 136)

wherein Xaa may be any amino acid, preferably a cysteine residue. A DNA sequence encoding the antigen identified as (g) above is provided in SEQ ID No. 52, and the polypeptide encoded by SEQ ID No. 52 is provided in SEQ ID No. 53. A DNA sequence encoding the antigen defined as (a) above is provided in SEQ ID No. 101; its deduced amino acid sequence is provided in SEQ ID No. 102. A DNA sequence corresponding to antigen (d) above is provided in SEQ ID No. 24 a DNA sequence corresponding to antigen (c) is provided in SEQ ID No. 25 and a DNA sequence corresponding to antigen (i) is provided in SEQ ID No. 99; its deduced amino acid sequence is provided in SEQ ID No. 100.

In a further specific embodiment, the subject invention discloses polypeptides comprising at least an immunogenic portion of an *M. tuberculosis* antigen having one of the following N-terminal sequences, or a variant thereof that differs only in conservative substitutions and/or modifications:

(m) Xaa-Tyr-Ile-Ala-Tyr-Xaa-Thr-Thr-Ala-Gly-Ile-Val-Pro-Gly-Lys-Ile-Asn-Val-His-Leu-Val; (SEQ ID No 137) or

(n) Asp-Pro-Pro-Asp-Pro-His-Gln-Xaa-Asp-Met-Thr-Lys-Gly-Tyr-Tyr-Pro-Gly-Gly-Arg-Arg-Xaa-Phe; (SEQ ID No. 129)

wherein Xaa may be any amino acid, preferably a cysteine residue.

In other specific embodiments, the subject invention discloses polypeptides comprising at least an immunogenic portion of a soluble *M. tuberculosis* antigen (or a variant of such an antigen) that comprises one or more of the amino acid sequences encoded by (a) the DNA sequences of SEQ ID Nos.: 1, 2, 4-10, 13-25 and 52; (b) the complements of such DNA sequences, or (c) DNA sequences substantially homologous to a sequence in (a) or (b).

In further specific embodiments, the subject invention discloses polypeptides comprising at least an immunogenic portion of a *M. tuberculosis* antigen (or a variant of such an antigen), which may or may not be soluble, that comprises one or more of the amino acid sequences encoded by (a) the DNA sequences of SEQ ID

Nos.: 26-51, 138, 139, 163-183 and 201, (b) the complements of such DNA sequences or (c) DNA sequences substantially homologous to a sequence in (a) or (b).

In the specific embodiments discussed above, the *M. tuberculosis* antigens include variants that are encoded by DNA sequences which are substantially homologous to one or more of DNA sequences specifically recited herein. "Substantial homology," as used herein, refers to DNA sequences that are capable of hybridizing under moderately stringent conditions. Suitable moderately stringent conditions include prewashing in a solution of 5X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5X SSC, overnight or, in the case of cross-species homology at 45°C, 0.5X SSC; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS). Such hybridizing DNA sequences are also within the scope of this invention, as are nucleotide sequences that, due to code degeneracy, encode an immunogenic polypeptide that is encoded by a hybridizing DNA sequence.

In a related aspect, the present invention provides fusion proteins comprising a first and a second inventive polypeptide or, alternatively, a polypeptide of the present invention and a known *M tuberculosis* antigen, such as the 38 kD antigen described in Andersen and Hansen, *Infect. Immun.* 57:2481-2488, 1989, (Genbank Accession No. M30046) or ESAT-6 (SEQ ID Nos. 103 and 104), together with variants of such fusion proteins. The fusion proteins of the present invention may also include a linker peptide between the first and second polypeptides.

A DNA sequence encoding a fusion protein of the present invention is constructed using known recombinant DNA techniques to assemble separate DNA sequences encoding the first and second polypeptides into an appropriate expression vector. The 3' end of a DNA sequence encoding the first polypeptide is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide so that the reading frames of the sequences are in phase to permit mRNA translation of the two DNA sequences into a single fusion protein that retains the biological activity of both the first and the second polypeptides.

A peptide linker sequence may be employed to separate the first and the second polypeptides by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion protein using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., *Gene 40*:39-46, 1985; Murphy et al., *Proc. Natl. Acad. Sci. USA 83*:8258-8262, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may be from 1 to about 50 amino acids in length. Peptide sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

The ligated DNA sequences are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of DNA are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons require to end translation and transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

In another aspect, the present invention provides methods for using one or more of the above polypeptides or fusion proteins (or DNA molecules encoding such polypeptides) to induce protective immunity against tuberculosis in a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may be afflicted with a disease, or may be free of detectable disease and/or infection. In other words, protective immunity may be induced to prevent or treat tuberculosis.

In this aspect, the polypeptide, fusion protein or DNA molecule is generally present within a pharmaceutical composition and/or a vaccine. Pharmaceutical compositions may comprise one or more polypeptides, each of which may contain one or more of the above sequences (or variants thereof), and a physiologically acceptable carrier. Vaccines may comprise one or more of the above polypeptides and a non-specific immune response enhancer, such as an adjuvant or a liposome (into which the polypeptide is incorporated). Such pharmaceutical compositions and vaccines may also contain other *M. tuberculosis* antigens, either incorporated into a combination polypeptide or present within a separate polypeptide.

Alternatively, a vaccine may contain DNA encoding one or more polypeptides as described above, such that the polypeptide is generated *in situ*. In such vaccines, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacterial and viral expression systems. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as *Bacillus-Calmette-Guerrin*) that expresses an immunogenic portion of the polypeptide on its cell surface. In a preferred embodiment, the DNA may be introduced using a viral expression system (*e.g.*, vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective), replication competent virus. Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, in Ulmer et al., *Science 259*:1745-1749, 1993 and reviewed by Cohen, *Science 259*:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

In a related aspect, a DNA vaccine as described above may be administered simultaneously with or sequentially to either a polypeptide of the present invention or a known *M. tuberculosis* antigen, such as the 38 kD antigen described above. For example, administration of DNA encoding a polypeptide of the present

invention, either "naked" or in a delivery system as described above, may be followed by administration of an antigen in order to enhance the protective immune effect of the vaccine.

Routes and frequency of administration, as well as dosage, will vary from individual to individual and may parallel those currently being used in immunization using BCG. In general, the pharmaceutical compositions and vaccines may be administered by injection (*e.g.*, intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally. Between 1 and 3 doses may be administered for a 1-36 week period. Preferably, 3 doses are administered, at intervals of 3-4 months, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of polypeptide or DNA that, when administered as described above, is capable of raising an immune response in an immunized patient sufficient to protect the patient from *M. tuberculosis* infection for at least 1-2 years. In general, the amount of polypeptide present in a dose (or produced *in situ* by the DNA in a dose) ranges from about 1 pg to about 100 mg per kg of host, typically from about 10 pg to about 1 mg, and preferably from about 100 pg to about 1 μg. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (*e.g.*, polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

Any of a variety of adjuvants may be employed in the vaccines of this invention to nonspecifically enhance the immune response. Most adjuvants contain a

substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a nonspecific stimulator of immune responses, such as lipid A, *Bortadella pertussis* or *Mycobacterium tuberculosis*. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Freund's Complete Adjuvant (Difco Laboratories) and Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ). Other suitable adjuvants include alum, biodegradable microspheres, monophosphoryl lipid A and quil A.

In another aspect, this invention provides methods for using one or more of the polypeptides described above to diagnose tuberculosis using a skin test. As used herein, a "skin test" is any assay performed directly on a patient in which a delayed-type hypersensitivity (DTH) reaction (such as swelling, reddening or dermatitis) is measured following intradermal injection of one or more polypeptides as described above. Such injection may be achieved using any suitable device sufficient to contact the polypeptide or polypeptides with dermal cells of the patient, such as a tuberculin syringe or 1 mL syringe. Preferably, the reaction is measured at least 48 hours after injection, more preferably 48-72 hours.

The DTH reaction is a cell-mediated immune response, which is greater in patients that have been exposed previously to the test antigen (*i.e.*, the immunogenic portion of the polypeptide employed, or a variant thereof). The response may be measured visually, using a ruler. In general, a response that is greater than about 0.5 cm in diameter, preferably greater than about 1.0 cm in diameter, is a positive response, indicative of tuberculosis infection, which may or may not be manifested as an active disease.

The polypeptides of this invention are preferably formulated, for use in a skin test, as pharmaceutical compositions containing a polypeptide and a physiologically acceptable carrier, as described above. Such compositions typically contain one or more of the above polypeptides in an amount ranging from about 1 μg to about 100 μg, preferably from about 10 μg to about 50 μg in a volume of 0.1 mL. Preferably, the carrier employed in such pharmaceutical compositions is a saline solution with appropriate preservatives, such as phenol and/or Tween 80™.

In a preferred embodiment, a polypeptide employed in a skin test is of sufficient size such that it remains at the site of injection for the duration of the reaction period. In general, a polypeptide that is at least 9 amino acids in length is sufficient. The polypeptide is also preferably broken down by macrophages within hours of injection to allow presentation to T-cells. Such polypeptides may contain repeats of one or more of the above sequences and/or other immunogenic or nonimmunogenic sequences.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

EXAMPLE 1

PURIFICATION AND CHARACTERIZATION OF POLYPEPTIDES FROM *M. TUBERCULOSIS* CULTURE FILTRATE

This example illustrates the preparation of *M. tuberculosis* soluble polypeptides from culture filtrate. Unless otherwise noted, all percentages in the following example are weight per volume.

M. tuberculosis (either H37Ra, ATCC No. 25177, or H37Rv, ATCC No. 25618) was cultured in sterile GAS media at 37°C for fourteen days. The media was then vacuum filtered (leaving the bulk of the cells) through a 0.45 μ filter into a sterile 2.5 L bottle. The media was next filtered through a 0.2 μ filter into a sterile 4 L bottle and NaN_3 was added to the culture filtrate to a concentration of 0.04%. The bottles were then placed in a 4°C cold room.

The culture filtrate was concentrated by placing the filtrate in a 12 L reservoir that had been autoclaved and feeding the filtrate into a 400 ml Amicon stir cell which had been rinsed with ethanol and contained a 10,000 kDa MWCO membrane.

The pressure was maintained at 60 psi using nitrogen gas. This procedure reduced the 12 L volume to approximately 50 ml.

The culture filtrate was dialyzed into 0.1% ammonium bicarbonate using a 8,000 kDa MWCO cellulose ester membrane, with two changes of ammonium bicarbonate solution. Protein concentration was then determined by a commercially available BCA assay (Pierce, Rockford, IL).

The dialyzed culture filtrate was then lyophilized, and the polypeptides resuspended in distilled water. The polypeptides were dialyzed against 0.01 mM 1,3 bis[tris(hydroxymethyl)-methylamino]propane, pH 7.5 (Bis-Tris propane buffer), the initial conditions for anion exchange chromatography. Fractionation was performed using gel profusion chromatography on a POROS 146 II Q/M anion exchange column 4.6 mm x 100 mm (Perseptive BioSystems, Framingham, MA) equilibrated in 0.01 mM Bis-Tris propane buffer pH 7.5. Polypeptides were eluted with a linear 0-0.5 M NaCl gradient in the above buffer system. The column eluent was monitored at a wavelength of 220 nm.

The pools of polypeptides eluting from the ion exchange column were dialyzed against distilled water and lyophilized. The resulting material was dissolved in 0.1% trifluoroacetic acid (TFA) pH 1.9 in water, and the polypeptides were purified on a Delta-Pak C18 column (Waters, Milford, MA) 300 Angstrom pore size, 5 micron particle size (3.9 x 150 mm). The polypeptides were eluted from the column with a linear gradient from 0-60% dilution buffer (0.1% TFA in acetonitrile). The flow rate was 0.75 ml/minute and the HPLC eluent was monitored at 214 nm. Fractions containing the eluted polypeptides were collected to maximize the purity of the individual samples. Approximately 200 purified polypeptides were obtained.

The purified polypeptides were then screened for the ability to induce T-cell proliferation in PBMC preparations. The PBMCs from donors known to be PPD skin test positive and whose T-cells were shown to proliferate in response to PPD and crude soluble proteins from MTB were cultured in medium comprising RPMI 1640 supplemented with 10% pooled human serum and 50 μg/ml gentamicin. Purified polypeptides were added in duplicate at concentrations of 0.5 to 10 μg/mL. After six

days of culture in 96-well round-bottom plates in a volume of 200 μl, 50 μl of medium was removed from each well for determination of IFN-γ levels, as described below. The plates were then pulsed with 1 μCi/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a gas scintillation counter. Fractions that resulted in proliferation in both replicates three fold greater than the proliferation observed in cells cultured in medium alone were considered positive.

IFN-γ was measured using an enzyme-linked immunosorbent assay (ELISA). ELISA plates were coated with a mouse monoclonal antibody directed to human IFN-γ (PharMingen, San Diego, CA) in PBS for four hours at room temperature. Wells were then blocked with PBS containing 5% (W/V) non-fat dried milk for 1 hour at room temperature. The plates were then washed six times in PBS/0.2% TWEEN-20 and samples diluted 1:2 in culture medium in the ELISA plates were incubated overnight at room temperature. The plates were again washed and a polyclonal rabbit anti-human IFN-γ serum diluted 1:3000 in PBS/10% normal goat serum was added to each well. The plates were then incubated for two hours at room temperature, washed and horseradish peroxidase-coupled anti-rabbit IgG (Sigma Chemical So., St. Louis, MO) was added at a 1:2000 dilution in PBS/5% non-fat dried milk. After a further two hour incubation at room temperature, the plates were washed and TMB substrate added. The reaction was stopped after 20 min with 1 N sulfuric acid. Optical density was determined at 450 nm using 570 nm as a reference wavelength. Fractions that resulted in both replicates giving an OD two fold greater than the mean OD from cells cultured in medium alone, plus 3 standard deviations, were considered positive.

For sequencing, the polypeptides were individually dried onto Biobrene™ (Perkin Elmer/Applied BioSystems Division, Foster City, CA) treated glass fiber filters. The filters with polypeptide were loaded onto a Perkin Elmer/Applied BioSystems Division Procise 492 protein sequencer. The polypeptides were sequenced from the amino terminal and using traditional Edman chemistry. The amino acid sequence was determined for each polypeptide by comparing the retention time of the PTH amino acid derivative to the appropriate PTH derivative standards.

Using the procedure described above, antigens having the following N-terminal sequences were isolated:

(a) Asp-Pro-Val-Asp-Ala-Val-Ile-Asn-Thr-Thr-Xaa-Asn-Tyr-Gly-Gln-Val-Val-Ala-Ala-Leu; (SEQ ID No. 54)

(b) Ala-Val-Glu-Ser-Gly-Met-Leu-Ala-Leu-Gly-Thr-Pro-Ala-Pro-Ser; (SEQ ID No. 55)

(c) Ala-Ala-Met-Lys-Pro-Arg-Thr-Gly-Asp-Gly-Pro-Leu-Glu-Ala-Ala-Lys-Glu-Gly-Arg; (SEQ ID No. 56)

(d) Tyr-Tyr-Trp-Cys-Pro-Gly-Gln-Pro-Phe-Asp-Pro-Ala-Trp-Gly-Pro; (SEQ ID No. 57)

(e) Asp-Ile-Gly-Ser-Glu-Ser-Thr-Glu-Asp-Gln-Gln-Xaa-Ala-Val; (SEQ ID No. 58)

(f) Ala-Glu-Glu-Ser-Ile-Ser-Thr-Xaa-Glu-Xaa-Ile-Val-Pro; (SEQ ID No. 59)

(g) Asp-Pro-Glu-Pro-Ala-Pro-Pro-Val-Pro-Thr-Ala-Ala-Ala-Ala-Pro-Pro-Ala; (SEQ ID No. 60) and

(h) Ala-Pro-Lys-Thr-Tyr-Xaa-Glu-Glu-Leu-Lys-Gly-Thr-Asp-Thr-Gly; (SEQ ID No. 61)

wherein Xaa may be any amino acid.

An additional antigen was isolated employing a microbore HPLC purification step in addition to the procedure described above. Specifically, 20 µl of a fraction comprising a mixture of antigens from the chromatographic purification step previously described, was purified on an Aquapore C18 column (Perkin Elmer/Applied Biosystems Division, Foster City, CA) with a 7 micron pore size, column size 1 mm x 100 mm, in a Perkin Elmer/Applied Biosystems Division Model 172 HPLC. Fractions were eluted from the column with a linear gradient of 1%/minute of acetonitrile (containing 0.05% TFA) in water (0.05% TFA) at a flow rate of 80 µl/minute. The eluent was monitored at 250 nm. The original fraction was separated into 4 major peaks plus other smaller components and a polypeptide was obtained which was shown to

have a molecular weight of 12.054 Kd (by mass spectrometry) and the following N-terminal sequence:

(i) Asp-Pro-Ala-Ser-Ala-Pro-Asp-Val-Pro-Thr-Ala-Ala-Gln-Gln-Thr-Ser-Leu-Leu-Asn-Asn-Leu-Ala-Asp-Pro-Asp-Val-Ser-Phe-Ala-Asp (SEQ ID No. 62).

This polypeptide was shown to induce proliferation and IFN-γ production in PBMC preparations using the assays described above.

Additional soluble antigens were isolated from *M. tuberculosis* culture filtrate as follows. *M. tuberculosis* culture filtrate was prepared as described above. Following dialysis against Bis-Tris propane buffer, at pH 5.5, fractionation was performed using anion exchange chromatography on a Poros QE column 4.6 x 100 mm (Perseptive Biosystems) equilibrated in Bis-Tris propane buffer pH 5.5. Polypeptides were eluted with a linear 0-1.5 M NaCl gradient in the above buffer system at a flow rate of 10 ml/min. The column eluent was monitored at a wavelength of 214 nm.

The fractions eluting from the ion exchange column were pooled and subjected to reverse phase chromatography using a Poros R2 column 4.6 x 100 mm (Perseptive Biosystems). Polypeptides were eluted from the column with a linear gradient from 0-100% acetonitrile (0.1% TFA) at a flow rate of 5 ml/min. The eluent was monitored at 214 nm.

Fractions containing the eluted polypeptides were lyophilized and resuspended in 80 µl of aqueous 0.1% TFA and further subjected to reverse phase chromatography on a Vydac C4 column 4.6 x 150 mm (Western Analytical, Temecula, CA) with a linear gradient of 0-100% acetonitrile (0.1% TFA) at a flow rate of 2 ml/min. Eluent was monitored at 214 nm.

The fraction with biological activity was separated into one major peak plus other smaller components. Western blot of this peak onto PVDF membrane revealed three major bands of molecular weights 14 Kd, 20 Kd and 26 Kd. These polypeptides were determined to have the following N-terminal sequences, respectively:

(j) Xaa-Asp-Ser-Glu-Lys-Ser-Ala-Thr-Ile-Lys-Val-Thr-Asp-Ala-Ser; (SEQ ID No. 134)

(k) Ala-Gly-Asp-Thr-Xaa-Ile-Tyr-Ile-Val-Gly-Asn-Leu-Thr-Ala-Asp; (SEQ ID No. 135) and

(l) Ala-Pro-Glu-Ser-Gly-Ala-Gly-Leu-Gly-Gly-Thr-Val-Gln-Ala-Gly; (SEQ ID No. 136), wherein Xaa may be any amino acid.

Using the assays described above, these polypeptides were shown to induce proliferation and IFN-γ production in PBMC preparations. Figs. 1A and B show the results of such assays using PBMC preparations from a first and a second donor, respectively.

DNA sequences that encode the antigens designated as (a), (c), (d) and (g) above were obtained by screening a genomic *M. tuberculosis* library using ^{32}P end labeled degenerate oligonucleotides corresponding to the N-terminal sequence and containing *M. tuberculosis* codon bias. The screen performed using a probe corresponding to antigen (a) above identified a clone having the sequence provided in SEQ ID No. 101. The polypeptide encoded by SEQ ID No. 101 is provided in SEQ ID No. 102. The screen performed using a probe corresponding to antigen (g) above identified a clone having the sequence provided in SEQ ID No. 52. The polypeptide encoded by SEQ ID No. 52 is provided in SEQ ID No. 53. The screen performed using a probe corresponding to antigen (d) above identified a clone having the sequence provided in SEQ ID No. 24, and the screen performed with a probe corresponding to antigen (c) identified a clone having the sequence provided in SEQ ID No: 25.

The above amino acid sequences were compared to known amino acid sequences in the gene bank using the DNA STAR system. The database searched contains some 173,000 proteins and is a combination of the Swiss, PIR databases along with translated protein sequences (Version 87). No significant homologies to the amino acid sequences for antigens (a)-(h) and (l) were detected.

The amino acid sequence for antigen (i) was found to be homologous to a sequence from *M. leprae*. The full length *M. leprae* sequence was amplified from genomic DNA using the sequence obtained from GENBANK. This sequence was then used to screen the *M. tuberculosis* library described below in Example 2 and a full length copy of the *M. tuberculosis* homologue was obtained (SEQ ID No. 99).

The amino acid sequence for antigen (j) was found to be homologous to a known *M. tuberculosis* protein translated from a DNA sequence. To the best of the inventors' knowledge, this protein has not been previously shown to possess T-cell stimulatory activity. The amino acid sequence for antigen (k) was found to be related to a sequence from *M. leprae*.

In the proliferation and IFN-γ assays described above, using three PPD positive donors, the results for representative antigens provided above are presented in Table 1:

TABLE 1

RESULTS OF PBMC PROLIFERATION AND IFN-γ ASSAYS

Sequence	Proliferation	IFN-γ
(a)	+	-
(c)	+++	+++
(d)	++	++
(g)	+++	+++
(h)	+++	+++

In Table 1, responses that gave a stimulation index (SI) of between 2 and 4 (compared to cells cultured in medium alone) were scored as +, an SI of 4-8 or 2-4 at a concentration of 1 μg or less was scored as ++ and an SI of greater than 8 was scored as +++. The antigen of sequence (i) was found to have a high SI (+++) for one donor and lower SI (++ and +) for the two other donors in both proliferation and IFN-γ assays. These results indicate that these antigens are capable of inducing proliferation and/or interferon-γ production.

EXAMPLE 2

USE OF PATIENT SERA TO ISOLATE *M. TUBERCULOSIS* ANTIGENS

This example illustrates the isolation of antigens from *M. tuberculosis* lysate by screening with serum from *M. tuberculosis*-infected individuals.

Dessicated *M. tuberculosis* H37Ra (Difco Laboratories) was added to a 2% NP40 solution, and alternately homogenized and sonicated three times. The resulting suspension was centrifuged at 13,000 rpm in microfuge tubes and the supernatant put through a 0.2 micron syringe filter. The filtrate was bound to Macro Prep DEAE beads (BioRad, Hercules, CA). The beads were extensively washed with 20 mM Tris pH 7.5 and bound proteins eluted with 1M NaCl. The 1M NaCl elute was dialyzed overnight against 10 mM Tris, pH 7.5. Dialyzed solution was treated with DNase and RNase at 0.05 mg/ml for 30 min. at room temperature and then with α-D-mannosidase, 0.5 U/mg at pH 4.5 for 3-4 hours at room temperature. After returning to pH 7.5, the material was fractionated via FPLC over a Bio Scale-Q-20 column (BioRad). Fractions were combined into nine pools, concentrated in a Centriprep 10 (Amicon, Beverley, MA) and then screened by Western blot for serological activity using a serum pool from *M. tuberculosis*-infected patients which was not immunoreactive with other antigens of the present invention.

The most reactive fraction was run in SDS-PAGE and transferred to PVDF. A band at approximately 85 Kd was cut out yielding the sequence:

(m) Xaa-Tyr-Ile-Ala-Tyr-Xaa-Thr-Thr-Ala-Gly-Ile-Val-Pro-Gly-Lys-Ile-Asn-Val-His-Leu-Val; (SEQ ID No. 137), wherein Xaa may be any amino acid.

Comparison of this sequence with those in the gene bank as described above, revealed no significant homologies to known sequences.

A DNA sequence that encodes the antigen designated as (m) above was obtained by screening a genomic *M. tuberculosis* Erdman strain library using labeled degenerate oligonucleotides corresponding to the N-terminal sequence of SEQ ID NO:137. A clone was identified having the DNA sequence provided in SEQ ID NO:

203. This sequence was found to encode the amino acid sequence provided in SEQ ID NO: 204. Comparison of these sequences with those in the genebank revealed some similarity to sequences previously identified in *M. tuberculosis* and *M. bovis*.

EXAMPLE 3

PREPARATION OF DNA SEQUENCES ENCODING *M. TUBERCULOSIS* ANTIGENS

This example illustrates the preparation of DNA sequences encoding *M. tuberculosis* antigens by screening a *M. tuberculosis* expression library with sera obtained from patients infected with *M. tuberculosis*, or with anti-sera raised against soluble *M. tuberculosis* antigens.

A. PREPARATION OF M. TUBERCULOSIS SOLUBLE ANTIGENS USING RABBIT ANTI-SERA RAISED AGAINST M. TUBERCULOSIS SUPERNATANT

Genomic DNA was isolated from the *M. tuberculosis* strain H37Ra. The DNA was randomly sheared and used to construct an expression library using the Lambda ZAP expression system (Stratagene, La Jolla, CA). Rabbit anti-sera was generated against secretory proteins of the *M. tuberculosis* strains H37Ra, H37Rv and Erdman by immunizing a rabbit with concentrated supernatant of the *M. tuberculosis* cultures. Specifically, the rabbit was first immunized subcutaneously with 200 μg of protein antigen in a total volume of 2 ml containing 10 μg muramyl dipeptide (Calbiochem, La Jolla, CA) and 1 ml of incomplete Freund's adjuvant. Four weeks later the rabbit was boosted subcutaneously with 100 μg antigen in incomplete Freund's adjuvant. Finally, the rabbit was immunized intravenously four weeks later with 50 μg protein antigen. The anti-sera were used to screen the expression library as described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989. Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the *M. tuberculosis* clones deduced.

Thirty two clones were purified. Of these, 25 represent sequences that have not been previously identified in human *M. tuberculosis*. Recombinant antigens

were expressed and purified antigens used in the immunological analysis described in Example 1. Proteins were induced by IPTG and purified by gel elution, as described in Skeiky et al., *J. Exp. Med. 181*:1527-1537, 1995. Representative sequences of DNA molecules identified in this screen are provided in SEQ ID Nos.: 1-25. The corresponding predicted amino acid sequences are shown in SEQ ID Nos. 63-87.

On comparison of these sequences with known sequences in the gene bank using the databases described above, it was found that the clones referred to hereinafter as TbRA2A, TbRA16, TbRA18, and TbRA29 (SEQ ID Nos. 76, 68, 70, 75) show some homology to sequences previously identified in *Mycobacterium leprae* but not in *M. tuberculosis*. TbRA11, TbRA26, TbRA28 and TbDPEP (SEQ ID Nos.: 65, 73, 74, 53) have been previously identified in *M. tuberculosis*. No significant homologies were found to TbRA1, TbRA3, TbRA4, TbRA9, TbRA10, TbRA13, TbRA17, TbRa19, TbRA29, TbRA32, TbRA36 and the overlapping clones TbRA35 and TbRA12 (SEQ ID Nos. 63, 77, 81, 82, 64, 67, 69, 71, 75, 78, 80, 79, 66). The clone TbRa24 is overlapping with clone TbRa29.

The results of PBMC proliferation and interferon-γ assays performed on representative recombinant antigens, and using T-cell preparations from several different *M. tuberculosis*-immune patients, are presented in Tables 2 and 3, respectively.

TABLE 2

RESULTS OF PBMC PROLIFERATION TO REPRESENTATIVE SOLUBLE ANTIGENS

Antigen	Patient												
	1	2	3	4	5	6	7	8	9	10	11	12	13
TbRa1	-	-	±	++	-	-	±	±	-	-	+	±	-
TbRa3	-	±	++	-	±	-	-	++	±	-	-	-	-
TbRa9	-	-	nt	nt	++	++	nt	nt	nt	nt	nt	nt	nt
TbRa10	-	-	±	±	±	+	nt	±	-	+	±	±	-
TbRa11	±	±	+	++	++	+	nt	-	++	++	++	±	nt
TbRa12	-	-	+	+	±	++	+	±	±	-	+	-	-
TbRa16	nt	nt	nt	nt	-	+	nt	nt	nt	nt	nt	nt	nt
TbRa24	nt	nt	nt	nt	-	-	nt	nt	nt	nt	nt	nt	nt
TbRa26	-	+	nt	nt	-	-	nt	nt	nt	nt	nt	nt	nt
TbRa29	nt	nt	nt	nt	-	-	nt	nt	nt	nt	nt	nt	nt
TbRa35	++	nt	++	++	++	++	nt	++	++	++	++	++	nt
TbRaB	nt	nt	nt	nt	-	-	nt	nt	nt	nt	nt	nt	nt
TbRaC	nt	nt	nt	nt	-	-	nt	nt	nt	nt	nt	nt	nt
TbRaD	nt	nt	nt	nt	-	-	nt	nt	nt	nt	nt	nt	nt
AAMK	-	-	±	-	-	-	nt	-	-	-	nt	±	nt
YY	-	-	-	-	-	-	nt	-	-	-	nt	+	nt
DPEP	-	+	-	++	-	-	nt	++	±	+	±	±	nt
Control	-	-	-	-	-	-	-	-	-	-	-	-	-

nt = not tested

TABLE 3

RESULTS OF PBMC INTERFERON-γ PRODUCTION TO REPRESENTATIVE SOLUBLE ANTIGENS

Antigen	Patient												
	1	2	3	4	5	6	7	8	9	10	11	12	13
TbRa1	+	++		+++	+	-		±	-	-	+	±	-
TbRa3	-	±	++	-	±	-	-	++	±	-	-	-	-
TbRa9	++	+	nt	nt	++	-	nt	nt	nt	nt	nt	nt	nt
TbRa10	+	+	±	±	±	+	nt	±	-	+	±	±	-
TbRa11		±	+	++	++	+	nt	-	++	++	++	±	nt
TbRa12	-	-	+	+	±	+++	+	±	±	-	+	-	-
TbRa16	nt	nt	nt	nt	+	+	nt	nt	nt	nt	nt	nt	nt
TbRa24	nt	nt	nt	nt	+	-	nt	nt	nt	nt	nt	nt	nt
TbRa26	++	++	nt	nt	+	+	nt	nt	nt	nt	nt	nt	nt
TbRa29	nt	nt	nt	nt	+	-	nt	nt	nt	nt	nt	nt	nt
TbRa35	++	nt	++	++	+++	+++	nt	++	++	+++	+++	++	nt
TbRaB	nt	nt	nt	nt	++	+	nt	nt	nt	nt	nt	nt	nt
TbRaC	nt	nt	nt	nt	+	+	nt	nt	nt	nt	nt	nt	nt
TbRaD	nt	nt	nt	nt	+	+	nt	nt	nt	nt	nt	nt	nt
AAMK	-	-	±	-	-	-	nt	-	-	-	nt	±	nt
YY	-	-	-	-	-	-	nt	-	-	-	nt	+	nt
DPEP	+	+	+	+++	+	-	nt	+++	±	+	±	±	nt
Control	-	-	-	-	-	-	-	-	-	-	-	-	-

In Tables 2 and 3, responses that gave a stimulation index (SI) of between 1.2 and 2 (compared to cells cultured in medium alone) were scored as ±, a SI of 2-4 was scored as +, as SI of 4-8 or 2-4 at a concentration of 1 μg or less was scored as ++ and an SI of greater than 8 was scored as +++. In addition, the effect of concentration on proliferation and interferon-γ production is shown for two of the above antigens in the attached Figure. For both proliferation and interferon-γ production, TbRa3 was scored as ++ and TbRa9 as +.

These results indicate that these soluble antigens can induce proliferation and/or interferon-γ production in T-cells derived from an *M. tuberculosis*-immune individual.

B. USE OF SERA FROM PATIENTS HAVING PULMONARY OR PLEURAL TUBERCULOSIS TO IDENTIFY DNA SEQUENCES ENCODING *M. TUBERCULOSIS* ANTIGENS

The genomic DNA library described above, and an additional H37Rv library, were screened using pools of sera obtained from patients with active tuberculosis. To prepare the H37Rv library, *M. tuberculosis* strain H37Rv genomic DNA was isolated, subjected to partial *Sau*3A digestion and used to construct an expression library using the Lambda Zap expression system (Stratagene, La Jolla, Ca). Three different pools of sera, each containing sera obtained from three individuals with active pulmonary or pleural disease, were used in the expression screening. The pools were designated TbL, TbM and TbH, referring to relative reactivity with H37Ra lysate (*i.e.*, TbL = low reactivity, TbM = medium reactivity and TbH = high reactivity) in both ELISA and immunoblot format. A fourth pool of sera from seven patients with active pulmonary tuberculosis was also employed. All of the sera lacked increased reactivity with the recombinant 38 kD *M. tuberculosis* H37Ra phosphate-binding protein.

All pools were pre-adsorbed with *E. coli* lysate and used to screen the H37Ra and H37Rv expression libraries, as described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989. Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the *M. tuberculosis* clones deduced.

Thirty two clones were purified. Of these, 31 represented sequences that had not been previously identified in human *M. tuberculosis*. Representative sequences of the DNA molecules identified are provided in SEQ ID Nos.: 26-51 and 105. Of these, TbH-8-2 (SEQ. ID NO. 105) is a partial clone of TbH-8, and TbH-4 (SEQ. ID NO. 43) and TbH-4-FWD (SEQ. ID NO. 44) are non-contiguous sequences from the same clone. Amino acid sequences for the antigens hereinafter identified as Tb38-1, TbH-4, TbH-8, TbH-9, and TbH-12 are shown in SEQ ID Nos.: 88-92. Comparison of these sequences with known sequences in the gene bank using the databases identified above revealed no significant homologies to TbH-4, TbH-8, TbH-9 and TbM-3, although weak homologies were found to TbH-9. TbH-12 was found to be homologous to a 34 kD antigenic protein previously identified in *M. paratuberculosis* (Acc. No. S28515). Tb38-1 was found to be located 34 base pairs upstream of the open reading frame for the antigen ESAT-6 previously identified in *M. bovis* (Acc. No. U34848) and in *M. tuberculosis* (Sorensen et al., *Infec. Immun. 63*:1710-1717, 1995).

Probes derived from Tb38-1 and TbH-9, both isolated from an H37Ra library, were used to identify clones in an H37Rv library. Tb38-1 hybridized to Tb38-1F2, Tb38-1F3, Tb38-1F5 and Tb38-1F6 (SEQ. ID NOS. 112, 113, 116, 118, and 119). (SEQ ID NOS. 112 and 113 are non-contiguous sequences from clone Tb38-1F2.) Two open reading frames were deduced in Tb38-IF2; one corresponds to Tb37FL (SEQ. ID. NO. 114), the second, a partial sequence, may be the homologue of Tb38-1 and is called Tb38-IN (SEQ. ID NO. 115). The deduced amino acid sequence of Tb38-1F3 is presented in SEQ. ID. NO. 117. A TbH-9 probe identified three clones in the H37Rv library: TbH-9-FL (SEQ. ID NO. 106), which may be the homologue of TbH-9 (R37Ra), TbH-9-1 (SEQ. ID NO. 108), and TbH-9-4 (SEQ. ID NO. 110), all of which are highly related sequences to TbH-9. The deduced amino acid sequences for these three clones are presented in SEQ ID NOS. 107, 109 and 111.

Further screening of the *M. tuberculosis* genomic DNA library, as described above, resulted in the recovery of ten additional reactive clones, representing seven different genes. One of these genes was identified as the 38 Kd antigen discussed

above, one was determined to be identical to the 14Kd alpha crystallin heat shock protein previously shown to be present in *M. tuberculosis*, and a third was determined to be identical to the antigen TbH-8 described above. The determined DNA sequences for the remaining five clones (hereinafter referred to as TbH-29, TbH-30, TbH-32 and TbH-33) are provided in SEQ ID NO: 138-141, respectively, with the corresponding predicted amino acid sequences being provided in SEQ ID NO: 142-145, respectively. The DNA and amino acid sequences for these antigens were compared with those in the gene bank as described above. No homologies were found to the 5' end of TbH-29 (which contains the reactive open reading frame), although the 3' end of TbH-29 was found to be identical to the *M. tuberculosis* cosmid Y227. TbH-32 and TbH-33 were found to be identical to the previously identified *M. tuberculosis* insertion element IS6110 and to the *M. tuberculosis* cosmid Y50, respectively. No significant homologies to TbH-30 were found.

Positive phagemid from this additional screening were used to infect *E. coli* XL-1 Blue MRF', as described in Sambrook et al., *supra*. Induction of recombinant protein was accomplished by the addition of IPTG. Induced and uninduced lysates were run in duplicate on SDS-PAGE and transferred to nitrocellulose filters. Filters were reacted with human *M. tuberculosis* sera (1:200 dilution) reactive with TbH and a rabbit sera (1:200 or 1:250 dilution) reactive with the N-terminal 4 Kd portion of lacZ. Sera incubations were performed for 2 hours at room temperature. Bound antibody was detected by addition of ^{125}I-labeled Protein A and subsequent exposure to film for variable times ranging from 16 hours to 11 days. The results of the immunoblots are summarized in Table 4.

TABLE 4

Antigen	Human M. tb Sera	Anti-lacZ Sera
TbH-29	45 Kd	45 Kd
TbH-30	No reactivity	29 Kd
TbH-32	12 Kd	12 Kd
TbH-33	16 Kd	16 Kd

Positive reaction of the recombinant human *M. tuberculosis* antigens with both the human *M. tuberculosis* sera and anti-lacZ sera indicate that reactivity of the human *M. tuberculosis* sera is directed towards the fusion protein. Antigens reactive with the anti-lacZ sera but not with the human *M. tuberculosis* sera may be the result of the human *M. tuberculosis* sera recognizing conformational epitopes, or the antigen-antibody binding kinetics may be such that the 2 hour sera exposure in the immunoblot is not sufficient.

The results of T-cell assays performed on Tb38-1, ESAT-6 and other representative recombinant antigens are presented in Tables 5A, B and 6, respectively, below:

TABLE 5A

RESULTS OF PBMC PROLIFERATION TO REPRESENTATIVE ANTIGENS

Antigen	Donor										
	1	2	3	4	5	6	7	8	9	10	11
Tb38.1	+++	+	-	-	-	++	-	+	-	++	+++
ESAT-6	+++	+	+	+	-	+	-	+	+	++	+++
TbH-9	++	++	-	++	±	±	++	++	++	++	++

TABLE 5B

RESULTS OF PBMC INTERFERON-γ PRODUCTION TO REPRESENTATIVE ANTIGENS

Antigen	Donor										
	1	2	3	4	5	6	7	8	9	10	11
Tb38.1	+++	+	-	+	+	+++	-	++	-	+++	+++
ESAT-6	+++	+	+	+	+-	+	-	+	+	+++	+++
TbH-9	++	++	-	+++	±	±	+++	+++	++	+++	++

TABLE 6

SUMMARY OF T-CELL RESPONSES TO REPRESENTATIVE ANTIGENS

Antigen	Proliferation			Interferon-γ			total
	patient 4	patient 5	patient 6	patient 4	patient 5	patient 6	
TbH9	++	++	++	+++	++	++	13
TbM7	-	+	-	++	+	-	4
TbH5	-	+	+	++	++	++	8
TbL23	-	+	±	++	++	+	7.5
TbH4	-	++	±	++	++	±	7
- control	-	-	-	-	-	-	0

These results indicate that both the inventive *M. tuberculosis* antigens and ESAT-6 can induce proliferation and/or interferon-γ production in T-cells derived from an *M. tuberculosis*-immune individual. To the best of the inventors' knowledge, ESAT-6 has not been previously shown to stimulate human immune responses

A set of six overlapping peptides covering the amino acid sequence of the antigen Tb38-1 was constructed using the method described in Example 6. The sequences of these peptides, hereinafter referred to as pep1-6, are provided in SEQ ID Nos. 93-98, respectively. The results of T-cell assays using these peptides are shown in Tables 7 and 8. These results confirm the existence, and help to localize T-cell epitopes within Tb38-1 capable of inducing proliferation and interferon-γ production in T-cells derived from an *M. tuberculosis* immune individual.

TABLE 7

RESULTS OF PBMC PROLIFERATION TO TB38-1 PEPTIDES

Peptide	Patient												
	1	2	3	4	5	6	7	8	9	10	11	12	13
pep1	-	-	-	-	±	-	-	-	-	±	-	-	+
pep2	±	-	-	-	±	-	-	-	±	±	-	-	+
pep3	-	-	-	-	-	-	-	-	±	-	-	-	±
pep4	++	-	-	-	-	-	+	-	±	±	-	-	+
pep5	++	±	-	-	-	-	+	-	±	-	-	-	+
pep6	-	++	-	-	-	-	±	-	±	+	-	-	+
Control	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 8

RESULTS OF PBMC INTERFERON-γ PRODUCTION TO TB38-1 PEPTIDES

Peptide	Patient												
	1	2	3	4	5	6	7	8	9	10	11	12	13
pep1	+	-	-	-	±	-	-	-	-	±	-	-	+
pep2		-	-	-	±	-	-	-	±	±	-	-	+
pep3	-	-	-	-	-	-	-	-	±	-	-	-	±
pep4	++	-	-	-	-	-	+	-	±	±	-	-	+
pep5	++	±	-	-	-	-	+	-	±	-	-	-	+
pep6	+	++	-	-	-	-	±	-	±	+	-	-	+
Control	-	-	-	-	-	-	-	-	-	-	-	-	-

Studies were undertaken to determine whether the antigens TbH-9 and Tb38-1 represent cellular proteins or are secreted into *M. tuberculosis* culture media. In the first study, rabbit sera were raised against A) secretory proteins of *M. tuberculosis*, B) the known secretory recombinant *M. tuberculosis* antigen 85b, C) recombinant Tb38-1 and D) recombinant TbH-9, using protocols substantially the same as that as described in Example 3A. Total *M. tuberculosis* lysate, concentrated supernatant of *M. tuberculosis* cultures and the recombinant antigens 85b, TbH-9 and Tb38-1 were resolved on denaturing gels, immobilized on nitrocellulose membranes and duplicate blots were probed using the rabbit sera described above.

The results of this analysis using control sera (panel I) and antisera (panel II) against secretory proteins, recombinant 85b, recombinant Tb38-1 and recombinant TbH-9 are shown in Figures 3A-D, respectively, wherein the lane designations are as follows: 1) molecular weight protein standards; 2) 5 μg of *M. tuberculosis* lysate; 3) 5 μg secretory proteins; 4) 50 ng recombinant Tb38-1; 5) 50 ng recombinant TbH-9; and 6) 50 ng recombinant 85b. The recombinant antigens were engineered with six terminal histidine residues and would therefore be expected to migrate with a mobility approximately 1 kD larger that the native protein. In Figure 3D, recombinant TbH-9 is lacking approximately 10 kD of the full-length 42 kD antigen, hence the significant difference in the size of the immunoreactive native TbH-9 antigen in the lysate lane (indicated by an arrow). These results demonstrate that Tb38-1 and TbH-9 are intracellular antigens and are not actively secreted by *M. tuberculosis*.

The finding that TbH-9 is an intracellular antigen was confirmed by determining the reactivity of TbH-9-specific human T cell clones to recombinant TbH-9, secretory *M. tuberculosis* proteins and PPD. A TbH-9-specific T cell clone (designated 131TbH-9) was generated from PBMC of a healthy PPD-positive donor. The proliferative response of 131TbH-9 to secretory proteins, recombinant TbH-9 and a control *M. tuberculosis* antigen, TbRa11, was determined by measuring uptake of tritiated thymidine, as described in Example 1. As shown in Figure 4A, the clone 131TbH-9 responds specifically to TbH-9, showing that TbH-9 is not a significant component of *M. tuberculosis* secretory proteins. Figure 4B shows the production of IFN-γ by a second TbH-9-specific T cell clone

(designated PPD 800-10) prepared from PBMC from a healthy PPD-positive donor, following stimulation of the T cell clone with secretory proteins, PPD or recombinant TbH-9. These results further confirm that TbH-9 is not secreted by *M. tuberculosis*.

C. USE OF SERA FROM PATIENTS HAVING EXTRAPULMONARY TUBERCULOSIS TO IDENTIFY DNA SEQUENCES ENCODING *M. TUBERCULOSIS* ANTIGENS

Genomic DNA was isolated from *M. tuberculosis* Erdman strain, randomly sheared and used to construct an expression library employing the Lambda ZAP expression system (Stratagene, La Jolla, CA). The resulting library was screened using pools of sera obtained from individuals with extrapulmonary tuberculosis, as described above in Example 3B, with the secondary antibody being goat anti-human IgG + A + M (H+L) conjugated with alkaline phosphatase.

Eighteen clones were purified. Of these, 4 clones (hereinafter referred to as XP14, XP24, XP31 and XP32) were found to bear some similarity to known sequences. The determined DNA sequences for XP14, XP24 and XP31 are provided in SEQ ID Nos.: 156-158, respectively, with the 5' and 3' DNA sequences for XP32 being provided in SEQ ID Nos.: 159 and 160, respectively. The predicted amino acid sequence for XP14 is provided in SEQ ID No: 161. The reverse complement of XP14 was found to encode the amino acid sequence provided in SEQ ID No.: 162.

Comparison of the sequences for the remaining 14 clones (hereinafter referred to as XP1-XP6, XP17-XP19, XP22, XP25, XP27, XP30 and XP36) with those in the genebank as described above, revealed no homologies with the exception of the 3' ends of XP2 and XP6 which were found to bear some homology to known *M. tuberculosis* cosmids. The DNA sequences for XP27 and XP36 are shown in SEQ ID Nos.: 163 and 164, respectively, with the 5' sequences for XP4, XP5, XP17 and XP30 being shown in SEQ ID Nos: 165-168, respectively, and the 5' and 3' sequences for XP2, XP3, XP6, XP18, XP19, XP22 and XP25 being shown in SEQ ID Nos: 169 and 170; 171 and 172; 173 and 174; 175 and 176; 177 and 178; 179 and 180; and 181 and 182, respectively. XP1 was found to overlap with the DNA sequences for TbH4, disclosed above. The full-length DNA sequence for TbH4-XP1 is provided in SEQ ID No.: 183. This DNA sequence was found to contain an

open reading frame encoding the amino acid sequence shown in SEQ ID No: 184. The reverse complement of TbH4-XP1 was found to contain an open reading frame encoding the amino acid sequence shown in SEQ ID No.: 185. The DNA sequence for XP36 was found to contain two open reading frames encoding the amino acid sequence shown in SEQ ID Nos.: 186 and 187, with the reverse complement containing an open reading frame encoding the amino acid sequence shown in SEQ ID No.: 188.

Recombinant XP1 protein was prepared as described above in Example 3B, with a metal ion affinity chromatography column being employed for purification. As illustrated in Figures 8A-B and 9A-B, using the assays described herein, recombinant XP1 was found to stimulate cell proliferation and IFN-γ production in T cells isolated from an *M. tuberculosis*-immune donors.

D. PREPARATION OF M. TUBERCULOSIS SOLUBLE ANTIGENS USING RABBIT ANTI-SERA RAISED AGAINST M. TUBERCULOSIS FRACTIONATED PROTEINS

M. tuberculosis lysate was prepared as described above in Example 2. The resulting material was fractionated by HPLC and the fractions screened by Western blot for serological activity with a serum pool from *M. tuberculosis*-infected patients which showed little or no immunoreactivity with other antigens of the present invention. Rabbit anti-sera was generated against the most reactive fraction using the method described in Example 3A . The anti-sera was used to screen an *M. tuberculosis* Erdman strain genomic DNA expression library prepared as described above. Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the *M. tuberculosis* clones determined.

Ten different clones were purified. Of these, one was found to be TbRa35, described above, and one was found to be the previously identified *M. tuberculosis* antigen, HSP60. Of the remaining eight clones, seven (hereinafter referred to as RDIF2, RDIF5, RDIF8, RDIF10, RDIF11 and RDIF 12) were found to bear some similarity to previously identified *M. tuberculosis* sequences. The determined DNA sequences for RDIF2, RDIF5, RDIF8, RDIF10 and RDIF11 are provided in SEQ ID Nos.: 189-193, respectively, with the corresponding predicted amino acid sequences being provided in SEQ ID Nos: 194-198, respectively. The 5' and 3' DNA sequences for RDIF12 are provided in SEQ ID Nos.: 199

and 200, respectively. No significant homologies were found to the antigen RDIF-7. The determined DNA and predicted amino acid sequences for RDIF7 are provided in SEQ ID Nos.: 201 and 202, respectively. One additional clone, referred to as RDIF6 was isolated, however, this was found to be identical to RDIF5.

Recombinant RDIF6, RDIF8, RDIF10 and RDIF11 were prepared as described above. As shown in Figures 8A-B and 9A-B, these antigens were found to stimulate cell proliferation and IFN-γ production in T cells isolated from *M. tuberculosis*-immune donors.

EXAMPLE 4

PURIFICATION AND CHARACTERIZATION OF A POLYPEPTIDE FROM TUBERCULIN PURIFIED PROTEIN DERIVATIVE

An *M. tuberculosis* polypeptide was isolated from tuberculin purified protein derivative (PPD) as follows.

PPD was prepared as published with some modification (Seibert, F. et al., Tuberculin purified protein derivative. Preparation and analyses of a large quantity for standard. The American Review of Tuberculosis 44:9-25, 1941).

M. tuberculosis Rv strain was grown for 6 weeks in synthetic medium in roller bottles at 37°C. Bottles containing the bacterial growth were then heated to 100° C in water vapor for 3 hours. Cultures were sterile filtered using a 0.22 μ filter and the liquid phase was concentrated 20 times using a 3 kD cut-off membrane. Proteins were precipitated once with 50% ammonium sulfate solution and eight times with 25% ammonium sulfate solution. The resulting proteins (PPD) were fractionated by reverse phase liquid chromatography (RP-HPLC) using a C18 column (7.8 x 300 mM; Waters, Milford, MA) in a Biocad HPLC system (Perseptive Biosystems, Framingham, MA). Fractions were eluted from the column with a linear gradient from 0-100% buffer (0.1% TFA in acetonitrile). The flow rate was 10 ml/minute and eluent was monitored at 214 nm and 280 nm.

Six fractions were collected, dried, suspended in PBS and tested individually in *M. tuberculosis*-infected guinea pigs for induction of delayed type hypersensitivity (DTH)

reaction. One fraction was found to induce a strong DTH reaction and was subsequently fractionated further by RP-HPLC on a microbore Vydac C18 column (Cat. No. 218TP5115) in a Perkin Elmer/Applied Biosystems Division Model 172 HPLC. Fractions were eluted with a linear gradient from 5-100% buffer (0.05% TFA in acetonitrile) with a flow rate of 80 µl/minute. Eluent was monitored at 215 nm. Eight fractions were collected and tested for induction of DTH in *M. tuberculosis*-infected guinea pigs. One fraction was found to induce strong DTH of about 16 mm induration. The other fractions did not induce detectable DTH. The positive fraction was submitted to SDS-PAGE gel electrophoresis and found to contain a single protein band of approximately 12 kD molecular weight.

This polypeptide, herein after referred to as DPPD, was sequenced from the amino terminal using a Perkin Elmer/Applied Biosystems Division Procise 492 protein sequencer as described above and found to have the N-terminal sequence shown in SEQ ID No.: 129. Comparison of this sequence with known sequences in the gene bank as described above revealed no known homologies. Four cyanogen bromide fragments of DPPD were isolated and found to have the sequences shown in SEQ ID Nos.: 130-133.

The ability of the antigen DPPD to stimulate human PBMC to proliferate and to produce IFN-γ was assayed as described in Example 1. As shown in Table 9, DPPD was found to stimulate proliferation and elicit production of large quantities of IFN-γ; more than that elicited by commercial PPD.

TABLE 9

RESULTS OF PROLIFERATION AND INTERFERON-γ ASSAYS TO DPPD

PBMC Donor	Stimulator	Proliferation (CPM)	IFN-γ (OD_{450})
A	Medium	1,089	0.17
	PPD (commercial)	8,394	1.29
	DPPD	13,451	2.21
B	Medium	450	0.09
	PPD (commercial)	3,929	1.26
	DPPD	6,184	1.49
C	Medium	541	0.11
	PPD (commercial)	8,907	0.76
	DPPD	23,024	>2.70

EXAMPLE 5

USE OF REPRESENTATIVE ANTIGENS FOR DIAGNOSIS OF TUBERCULOSIS

This example illustrates the effectiveness of several representative polypeptides in skin tests for the diagnosis of *M. tuberculosis* infection.

Individuals were injected intradermally with 100 µl of either PBS or PBS plus Tween 20™ containing either 0.1 µg of protein (for TbH-9 and TbRa35) or 1.0 µg of protein (for TbRa38-1). Induration was measured between 5-7 days after injection, with a response of 5 mm or greater being considered positive. Of the 20 individuals tested, 2 were PPD negative and 18 were PPD positive. Of the PPD positive individuals, 3 had active tuberculosis, 3 had been previously infected with tuberculosis and 9 were healthy. In a second study, 13 PPD positive individuals were tested with 0.1 µg TbRa11 in either PBS or PBS plus Tween 20™ as described above. The results of both studies are shown in Table 10.

TABLE 10

RESULTS OF DTH TESTING WITH REPRESENTATIVE ANTIGENS

	TbH-9 Pos/Total	Tb38-1 Pos/Total	TbRa35 Pos/Total	Cumulative Pos/Total	TbRa11 Pos/Total
PPD negative	0/2	0/2	0/2	0/2	
PPD positive					
healthy	5/9	4/9	4/9	6/9	1/4
prior TB	3/5	2/5	2/5	4/5	3/5
active	3/4	3/4	0/4	4/4	1/4
TOTAL	11/18	9/18	6/18	14/18	5/13

EXAMPLE 6

SYNTHESIS OF SYNTHETIC POLYPEPTIDES

Polypeptides may be synthesized on a Millipore 9050 peptide synthesizer using FMOC chemistry with HPTU (O-Benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate) activation. A Gly-Cys-Gly sequence may be attached to the amino terminus of the peptide to provide a method of conjugation or labeling of the peptide. Cleavage of the peptides from the solid support may be carried out using the following cleavage mixture: trifluoroacetic acid:ethanedithiol:thioanisole:water:phenol (40:1:2:2:3). After cleaving for 2 hours, the peptides may be precipitated in cold methyl-t-butyl-ether. The peptide pellets may then be dissolved in water containing 0.1% trifluoroacetic acid (TFA) and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0%-60% acetonitrile (containing 0.1% TFA) in water (containing 0.1% TFA) may be used to elute the peptides. Following lyophilization of the pure fractions, the peptides may be characterized using electrospray mass spectrometry and by amino acid analysis.

EXAMPLE 7

PREPARATION AND CHARACTERIZATION OF *M. TUBERCULOSIS* FUSION PROTEINS

A fusion protein containing TbRa3, the 38 kD antigen and Tb38-1 was prepared as follows.

Each of the DNA constructs TbRa3, 38 kD and Tb38-1 were modified by PCR in order to facilitate their fusion and the subsequent expression of the fusion protein TbRa3-38 kD-Tb38-1. TbRa3, 38 kD and Tb38-1 DNA was used to perform PCR using the primers PDM-64 and PDM-65 (SEQ ID NO: 146 and 147), PDM-57 and PDM-58 (SEQ ID NO: 148 and 149), and PDM-69 and PDM-60 (SEQ ID NO: 150 and 151), respectively. In each case, the DNA amplification was performed using 10 µl 10X Pfu buffer, 2 µl 10 mM dNTPs, 2 µl each of the PCR primers at 10 µM concentration, 81.5 µl water, 1.5 µl Pfu DNA polymerase (Stratagene, La Jolla, CA) and 1 µl DNA at either 70 ng/µl (for TbRa3) or 50 ng/µl (for 38 kD and Tb38-1). For TbRa3, denaturation at 94°C was performed for 2 min, followed by 40 cycles of 96°C for 15 sec and 72°C for 1 min, and lastly by 72°C for 4 min. For 38 kD, denaturation at 96°C was performed for 2 min, followed by 40 cycles of 96°C for 30 sec, 68°C for 15 sec and 72°C for 3 min, and finally by 72°C for 4 min. For Tb38-1 denaturation at 94°C for 2 min was followed by 10 cycles of 96°C for 15 sec, 68°C for 15 sec and 72°C for 1.5 min, 30 cycles of 96°C for 15 sec, 64°C for 15 sec and 72°C for 1.5, and finally by 72°C for 4 min.

The TbRa3 PCR fragment was digested with NdeI and EcoRI and cloned directly into pT7^L2 IL 1 vector using NdeI and EcoRI sites. The 38 kD PCR fragment was digested with Sse8387I, treated with T4 DNA polymerase to make blunt ends and then digested with EcoRI for direct cloning into the pT7^L2Ra3-1 vector which was digested with StuI and EcoRI. The 38-1 PCR fragment was digested with Eco47III and EcoRI and directly subcloned into pT7^L2Ra3/38kD-17 digested with the same enzymes. The whole fusion was then transferred to pET28b – using NdeI and EcoRI sites. The fusion construct was confirmed by DNA sequencing.

The expression construct was transformed into BLR pLys S *E. coli* (Novagen, Madison, WI) and grown overnight in LB broth with kanamycin (30 µg/ml) and chloramphenicol (34 µg/ml). This culture (12 ml) was used to inoculate 500 ml 2XYT with

the same antibiotics and the culture was induced with IPTG at an OD560 of 0.44 to a final concentration of 1.2 mM. Four hours post-induction, the bacteria were harvested and sonicated in 20 mM Tris (8.0), 100 mM NaCl, 0.1% DOC, 20 μg/ml Leupeptin, 20 mM PMSF followed by centrifugation at 26,000 X g. The resulting pellet was resuspended in 8 M urea, 20 mM Tris (8.0), 100 mM NaCl and bound to Pro-bond nickel resin (Invitrogen, Carlsbad, CA). The column was washed several times with the above buffer then eluted with an imidazole gradient (50 mM, 100 mM, 500 mM imidazole was added to 8 M urea, 20 mM Tris (8.0), 100 mM NaCl). The eluates containing the protein of interest were then dialzyed against 10 mM Tris (8.0).

The DNA and amino acid sequences for the resulting fusion protein (hereinafter referred to as TbRa3-38 kD-Tb38-1) are provided in SEQ ID NO: 152 and 153, respectively.

A fusion protein containing the two antigens TbH-9 and Tb38-1 (hereinafter referred to as TbH9-Tb38-1) without a hinge sequence, was prepared using a similar procedure to that described above. The DNA sequence for the TbH9-Tb38-1 fusion protein is provided in SEQ ID NO: 156.

The ability of the fusion protein TbH9-Tb38-1 to induce T cell proliferation and IFN-γ production in PBMC preparations was examined using the protocol described above in Example 1. PBMC from three donors were employed: one who had been previously shown to respond to TbH9 but not Tb38-1 (donor 131); one who had been shown to respond to Tb38-1 but not TbH9 (donor 184); and one who had been shown to respond to both antigens (donor 201). The results of these studies (Figs. 5-7, respectively) demonstrate the functional activity of both the antigens in the fusion protein.

A fusion protein containing TbRa3, the antigen 38kD, Tb38-1 and DPEP was prepared as follows.

Each of the DNA constructs TbRa3, 38 kD and Tb38-1 were modified by PCR and cloned into vectors essentially as described above, with the primers PDM-69 (SEQ ID NO:150 and PDM-83 (SEQ ID NO: 205) being used for amplification of the Tb38-1A fragment. Tb38-1A differs from Tb38-1 by a DraI site at the 3' end of the coding region that

keeps the final amino acid intact while creating a blunt restriction site that is in frame. The TbRa3/38kD/Tb38-1A fusion was then transferred to pET28b using NdeI and EcoR1 sites.

DPEP DNA was used to perform PCR using the primers PDM-84 and PDM-85 (SEQ ID NO: 206 and 207, respectively) and 1 µl DNA at 50 ng/µl. Denaturation at 94 °C was performed for 2 min, followed by 10 cycles of 96 °C for 15 sec, 68 °C for 15 sec and 72 °C for 1.5 min; 30 cycles of 96 °C for 15 sec, 64 °C for 15 sec and 72 °C for 1.5 min; and finally by 72 °C for 4 min. The DPEP PCR fragment was digested with EcoRI and Eco72I and clones directly into the pET28Ra3/38kD/38-1A construct which was digested with DraI and EcoRI. The fusion construct was confirmed to be correct by DNA sequencing. Recombinant protein was prepared as described above. The DNA and amino acid sequences for the resulting fusion protein (hereinafter referred to as TbF-2) are provided in SEQ ID NO: 208 and 209, respectively.

The reactivity of the fusion protein TbF-2 with sera from *M. tuberculosis*-infected patients was examined by ELISA using the protocol described above. The results of these studies (Table 11) demonstrate that all four antigens function independently in the fusion protein.

TABLE 11

REACTIVITY OF TBF-2 FUSION RECOMBINANT WITH TB AND NORMAL SERA

Serum ID	Status	TbF OD450	Status	TbF-2 OD450	Status	ELISA Reactivity			
						38 kD	TbRa3	Tb38-1	DPEP
B931-40	TB	0.57	+	0.321	+	-	+	-	+
B931-41	TB	0.601	+	0.396	+	+	+	+	-
B931-109	TB	0.494	+	0.404	+	+	+	±	-
B931-132	TB	1.502	+	1.292	+	+	+	+	±
5004	TB	1.806	+	1.666	+	±	±	+	-
15004	TB	2.862	+	2.468	+	+	+	+	-
39004	TB	2.443	+	1.722	+	+	+	+	-
68004	TB	2.871	+	2.575	+	+	+	+	-
99004	TB	0.691	+	0.971	+	-	±	+	-
107004	TB	0.875	+	0.732	+	-	±	+	-
92004	TB	1.632	+	1.394	+	+	±	±	-
97004	TB	1.491	+	1.979	+	+	±	-	+
118004	TB	3.182	+	3.045	+	+	±	-	-
173004	TB	3.644	+	3.578	+	+	+	+	-
175004	TB	3.332	+	2.916	+	+	+	-	-
274004	TB	3.696	+	3.716	+	-	+	-	+
276004	TB	3.243	+	2.56	+	-	-	+	-
282004	TB	1.249	+	1.234	+	+	-	-	-
289004	TB	1.373	+	1.17	+	-	+	-	-
308004	TB	3.708	+	3.355	+	-	-	+	-
314004	TB	1.663	+	1.399	+	-	-	+	-
317004	TB	1.163	+	0.92	+	+	-	-	-
312004	TB	1.709	+	1.453	+	-	+	-	-
380004	TB	0.238	-	0.461	+	-	±	-	+
451004	TB	0.18	-	0.2	-	-	-	-	±
478004	TB	0.188	-	0.469	+	-	-	-	±
410004	TB	0.384	+	2.392	+	±	-	-	+
411004	TB	0.306	+	0.874	+	-	+	-	+
421004	TB	0.357	+	1.456	+	-	+	-	+
528004	TB	0.047	-	0.196	-	-	-	-	+
A6-87	Normal	0.094	-	0.063	-	-	-	-	-
A6-88	Normal	0.214	-	0.19	-	-	-	-	-
A6-89	Normal	0.248	-	0.125	-	-	-	-	-
A6-90	Normal	0.179	-	0.206	-	-	-	-	-
A6-91	Normal	0.135	-	0.151	-	-	-	-	-
A6-92	Normal	0.064	-	0.097	-	-	-	-	-
A6-93	Normal	0.072	-	0.098	-	-	-	-	-
A6-94	Normal	0.072	-	0.064	-	-	-	-	-
A6-95	Normal	0.125	-	0.159	-	-	-	-	-
A6-96	Normal	0.121	-	0.12	-	-	-	-	-
Cut-off		0.284		0.266					

One of skill in the art will appreciate that the order of the individual antigens within the fusion protein may be changed and that comparable activity would be expected provided each of the epitopes is still functionally available. In addition, truncated forms of the proteins containing active epitopes may be used in the construction of fusion proteins.

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From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS: Reed, Steven G.
Skeiky, Yasir A.W.
Dillon, Davin C.
Campos-Neto, Antonio
Houghton, Raymond
Vedvick, Thomas S.
Twardzik, Daniel R.
Lodes, Michael J.

(ii) TITLE OF INVENTION: COMPOUNDS AND METHODS FOR IMMUNOTHERAPY AND DIAGNOSIS OF TUBERCULOSIS

(iii) NUMBER OF SEQUENCES: 214

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: SEED and BERRY LLP
(B) STREET: 6300 Columbia Center, 701 Fifth Avenue
(C) CITY: Seattle
(D) STATE: Washington
(E) COUNTRY: USA
(F) ZIP: 98104-7092

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

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(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Maki, David J.
(B) REGISTRATION NUMBER: 31,392
(C) REFERENCE/DOCKET NUMBER: 210121.411C7

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (206) 622-4900
(B) TELEFAX: (206) 682-6031

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 766 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AGAGCCATGT GACGGTAGTT GCGGTGCTCG GGGTACTCGG CGTATTTCTG ATGGTCTCGG 420

CGACGTTTAA CAAGCCCAGC GCCTATTCGA CCGGTTGGGC ATTGTGGGTT GTGTTGGCTT 480

TCATCGTGTT CCAGGCGGTT GCGGCAGTCC TGGCGCTCTT GGTGGAGACC GGCGCTATCA 540

CCGCGCCGGC GCCGCGGCCC AAGTTCGACC CGTATGGACA GTACGGGCGG TACGGGCAGT 600

ACGGGCAGTA CGGGGTGCAG CCGGGTGGGT ACTACGGTCA GCAGGGTGCT CAGCAGGCCG 660

CGGGACTGCA GTCGCCCGGC CCGCAGCAGT CTCCGCAGCC TCCCGGATAT GGGTCGCAGT 720

ACGGCGGCTA TTCGTCCAGT CCGAGCCAAT CGGGCAGTGG ATACACTGCT CAGCCCCCGG 780

CCCAGCCGCC GGCGCAGTCC GGGTCGCAAC AATCGCACCA GGGCCCATCC ACGCCACCTA 840

CCGGCTTTCC GAGCTTCAGC CCACCACCAC CGGTCAGTGC CGGGACGGGG TCGCAGGCTG 900

GTTCGGCTCC AGTCAACTAT TCAAACCCCA GCGGGGGCGA GCAGTCGTCG TCCCCCGGGG 960

GGGCGCCGGT CTAACCGGGC GTTCCCGCGT CCGGTCGCGC GTGTGCGCGA AGAGTGAACA 1020

GGGTGTCAGC AAGCGCGGAC GATCCTCGTG CCGAATTC 1058

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 327 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

CGGCACGAGA GACCGATGCC GCTACCCTCG CGCAGGAGGC AGGTAATTTC GAGCGGATCT 60

CCGGCGACCT GAAAACCCAG ATCGACCAGG TGGAGTCGAC GGCAGGTTCG TTGCAGGGCC 120

AGTGGCGCGG CGCGGCGGGG ACGGCCGCCC AGGCCGCGGT GGTGCGCTTC CAAGAAGCAG 180

CCAATAAGCA GAAGCAGGAA CTCGACGAGA TCTCGACGAA TATTCGTCAG GCCGGCGTCC 240

AATACTCGAG GGCCGACGAG GAGCAGCAGC AGGCGCTGTC CTCGCAAATG GGCTTCTGAC 300

CCGCTAATAC GAAAAGAAAC GGAGCAA 327

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 170 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

CGGTCGCGAT GATGGCGTTG TCGAACGTGA CCGATTCTGT ACCGCCGTCG TTGAGATCAA 60

CCAACAACGT GTTGGCGTCG GCAAATGTGC CGNACCCGTG GATCTCGGTG ATCTTGTTCT 120

TCTTCATCAG GAAGTGCACA CCGGCCACCC TGCCCTCGGN TACCTTTCGG 170

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 127 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GATCCGGCGG CACGGGGGGT GCCGGCGGCA GCACCGCTGG CGCTGGCGGC AACGGCGGGG 60

CCGGGGGTGG CGGCGGAACC GGTGGGTTGC TCTTCGGCAA CGGCGGTGCC GGCGGGCACG 120

GGGCCGT 127

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CGGCGGCAAG GGCGGCACCG CCGGCAACGG GAGCGGCGCG GCCGGCGGCA ACGGCGGCAA 60

CGGCGGCTCC GGCCTCAACG G 81

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 149 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GATCAGGGCT GGCCGGCTCC GGCCAGAAGG GCGGTAACGG AGGAGCTGCC GGATTGTTTG 60

85 90 95

Pro Ala Ala Gly Gly Gly Ala
100

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 88 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

Val Gln Cys Arg Val Trp Leu Glu Ile Gln Trp Arg Gly Met Leu Gly
1 5 10 15

Ala Asp Gln Ala Arg Ala Gly Gly Pro Ala Arg Ile Trp Arg Glu His
20 25 30

Ser Met Ala Ala Met Lys Pro Arg Thr Gly Asp Gly Pro Leu Glu Ala
35 40 45

Thr Lys Glu Gly Arg Gly Ile Val Met Arg Val Pro Leu Glu Gly Gly
50 55 60

Gly Arg Leu Val Val Glu Leu Thr Pro Asp Glu Ala Ala Ala Leu Gly
65 70 75 80

Asp Glu Leu Lys Gly Val Thr Ser
85

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 95 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

Thr Asp Ala Ala Thr Leu Ala Gln Glu Ala Gly Asn Phe Glu Arg Ile
1 5 10 15

Ser Gly Asp Leu Lys Thr Gln Ile Asp Gln Val Glu Ser Thr Ala Gly
20 25 30

Ser Leu Gln Gly Gln Trp Arg Gly Ala Ala Gly Thr Ala Ala Gln Ala
35 40 45

Ala Val Val Arg Phe Gln Glu Ala Ala Asn Lys Gln Lys Gln Glu Leu
50 55 60

Asp Glu Ile Ser Thr Asn Ile Arg Gln Ala Gly Val Gln Tyr Ser Arg
65 70 75 80

Ala Asp Glu Glu Gln Gln Gln Ala Leu Ser Ser Gln Met Gly Phe
85 90 95

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 166 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

Met Thr Gln Ser Gln Thr Val Thr Val Asp Gln Gln Glu Ile Leu Asn
1 5 10 15

Arg Ala Asn Glu Val Glu Ala Pro Met Ala Asp Pro Pro Thr Asp Val
20 25 30

Pro Ile Thr Pro Cys Glu Leu Thr Xaa Xaa Lys Asn Ala Ala Gln Gln
35 40 45

Xaa Val Leu Ser Ala Asp Asn Met Arg Glu Tyr Leu Ala Ala Gly Ala
50 55 60

Lys Glu Arg Gln Arg Leu Ala Thr Ser Leu Arg Asn Ala Ala Lys Xaa
65 70 75 80

Tyr Gly Glu Val Asp Glu Glu Ala Ala Thr Ala Leu Asp Asn Asp Gly
85 90 95

Glu Gly Thr Val Gln Ala Glu Ser Ala Gly Ala Val Gly Gly Asp Ser
100 105 110

Ser Ala Glu Leu Thr Asp Thr Pro Arg Val Ala Thr Ala Gly Glu Pro
115 120 125

Asn Phe Met Asp Leu Lys Glu Ala Ala Arg Lys Leu Glu Thr Gly Asp
130 135 140

Gln Gly Ala Ser Leu Ala His Xaa Gly Asp Gly Trp Asn Thr Xaa Thr
145 150 155 160

Leu Thr Leu Gln Gly Asp
165

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

Gln Gln Gly Ala Gln Gln Ala Ala Gly Leu Gln Ser Pro Gly Pro Gln
195 200 205

Gln Ser Pro Gln Pro Pro Gly Tyr Gly Ser Gln Tyr Gly Gly Tyr Ser
210 215 220

Ser Ser Pro Ser Gln Ser Gly Ser Gly Tyr Thr Ala Gln Pro Pro Ala
225 230 235 240

Gln Pro Pro Ala Gln Ser Gly Ser Gln Gln Ser His Gln Gly Pro Ser
245 250 255

Thr Pro Pro Thr Gly Phe Pro Ser Phe Ser Pro Pro Pro Pro Val Ser
260 265 270

Ala Gly Thr Gly Ser Gln Ala Gly Ser Ala Pro Val Asn Tyr Ser Asn
275 280 285

Pro Ser Gly Gly Glu Gln Ser Ser Ser Pro Gly Gly Ala Pro Val
290 295 300

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

Gly Cys Gly Glu Thr Asp Ala Ala Thr Leu Ala Gln Glu Ala Gly Asn
1 5 10 15

Phe Glu Arg Ile Ser Gly Asp Leu Lys Thr Gln Ile
20 25

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

Asp Gln Val Glu Ser Thr Ala Gly Ser Leu Gln Gly Gln Trp Arg Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

Gly Cys Gly Ser Thr Ala Gly Ser Leu Gln Gly Gln Trp Arg Gly Ala
1 5 10 15

Ala Gly Thr Ala Ala Gln Ala Ala Val Val Arg
20 25

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

Gly Cys Gly Gly Thr Ala Ala Gln Ala Ala Val Val Arg Phe Gln Glu
1 5 10 15

Ala Ala Asn Lys Gln Lys Gln Glu Leu Asp Glu
20 25

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

Gly Cys Gly Ala Asn Lys Gln Lys Gln Glu Leu Asp Glu Ile Ser Thr
1 5 10 15

Asn Ile Arg Gln Ala Gly Val Gln Tyr Ser Arg
20 25

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

Gly Cys Gly Ile Arg Gln Ala Gly Val Gln Tyr Ser Arg Ala Asp Glu
1 5 10 15

Glu Gln Gln Gln Ala Leu Ser Ser Gln Met Gly Phe
20 25

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 507 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

ATGAAGATGG TGAAATCGAT CGCCGCAGGT CTGACCGCCG CGGCTGCAAT CGGCGCCGCT 60

GCGGCCGGTG TGACTTCGAT CATGGCTGGC GGCCCGGTCG TATACCAGAT GCAGCCGGTC 120

GTCTTCGGCG CGCCACTGCC GTTGGACCCG GCATCCGCCC CTGACGTCCC GACCGCCGCC 180

CAGTTGACCA GCCTGCTCAA CAGCCTCGCC GATCCCAACG TGTCGTTTGC GAACAAGGGC 240

AGTCTGGTCG AGGGCGGCAT CGGGGGCACC GAGGCGCGCA TCGCCGACCA CAAGCTGAAG 300

AAGGCCGCCG AGCACGGGGA TCTGCCGCTG TCGTTCAGCG TGACGAACAT CCAGCCGGCG 360

GCCGCCGGTT CGGCCACCGC CGACGTTTCC GTCTCGGGTC CGAAGCTCTC GTCGCCGGTC 420

ACGCAGAACG TCACGTTCGT GAATCAAGGC GGCTGGATGC TGTCACGCGC ATCGGCGATG 480

GAGTTGCTGC AGGCCGCAGG GAACTGA 507

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 168 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

```
     130                 135                 140

Trp Ala Gln Asp Ala Ala Ala Met Phe Gly Tyr Ala Ala Thr Ala Ala
145                 150                 155                 160

Thr Ala Thr Glu Ala Leu Leu Pro Phe Glu Asp Ala Pro Leu Ile Thr
                165                 170                 175

Asn Pro Gly Gly Leu Leu Glu Gln Ala Val Ala Val Glu Glu Ala Ile
            180                 185                 190

Asp Thr Ala Ala Ala Asn Gln Leu Met Asn Asn Val Pro Gln Ala Leu
        195                 200                 205

Gln Gln Leu Ala Gln Pro Thr Lys Ser Ile Trp Pro Phe Asp Gln Leu
    210                 215                 220

Ser Glu Leu Trp Lys Ala Ile Ser Pro His Leu Ser Pro Leu Ser Asn
225                 230                 235                 240

Ile Val Ser Met Leu Asn Asn His Val Ser Met Thr Asn Ser Gly Val
                245                 250                 255

Ser Met Ala Ser Thr Leu His Ser Met Leu Lys Gly Phe Ala Pro Ala
            260                 265                 270

Ala Ala Gln Ala Val Glu Thr Ala Ala Gln Asn Gly Val Gln Ala Met
        275                 280                 285

Ser Ser Leu Gly Ser Gln Leu Gly Ser Ser Leu Gly Ser Ser Gly Leu
    290                 295                 300

Gly Ala Gly Val Ala Ala Asn Leu Gly Arg Ala Ala Ser Val Gly Ser
305                 310                 315                 320

Leu Ser Val Pro Gln Ala Trp Ala Ala Ala Asn Gln Ala Val Thr Pro
                325                 330                 335

Ala Ala Arg Ala Leu Pro Leu Thr Ser Leu Thr Ser Ala Ala Gln Thr
            340                 345                 350

Ala Pro Gly His Met Leu Gly Gly Leu Pro Leu Gly Gln Leu Thr Asn
        355                 360                 365

Ser Gly Gly Gly Phe Gly Gly Val Ser Asn Ala Leu Arg Met Pro Pro
    370                 375                 380

Arg Ala Tyr Val Met Pro Arg Val Pro Ala Ala Gly
385                 390                 395
```

(2) INFORMATION FOR SEQ ID NO:112:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1616 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

CATCGGAGGG AGTGATCACC ATGCTGTGGC ACGCAATGCC ACCGGAGTAA ATACCGCACG 60

GCTGATGGCC GGCGCGGGTC CGGCTCCAAT GCTTGCGGCG GCCGCGGGAT GGCAGACGCT 120

TTCGGCGGCT CTGGACGCTC AGGCCGTCGA GTTGACCGCG CGCCTGAACT CTCTGGGAGA 180

AGCCTGGACT GGAGGTGGCA GCGACAAGGC GCTTGCGGCT GCAACGCCGA TGGTGGTCTG 240

GCTACAAACC GCGTCAACAC AGGCCAAGAC CCGTGCGATG CAGGCGACGG CGCAAGCCGC 300

GGCATACACC CAGGCCATGG CCACGACGCC GTCGCTGCCG GAGATCGCCG CCAACCACAT 360

CACCCAGGCC GTCCTTACGG CCACCAACTT CTTCGGTATC AACACGATCC CGATCGCGTT 420

GACCGAGATG GATTATTTCA TCCGTATGTG GAACCAGGCA GCCCTGGCAA TGGAGGTCTA 480

CCAGGCCGAG ACCGCGGTTA ACACGCTTTT CGAGAAGCTC GAGCCGATGG CGTCGATCCT 540

TGATCCCGGC GCGAGCCAGA GCACGACGAA CCCGATCTTC GGAATGCCCT CCCCTGGCAG 600

CTCAACACCG GTTGGCCAGT TGCCGCCGGC GGCTACCCAG ACCCTCGGCC AACTGGGTGA 660

GATGAGCGGC CCGATGCAGC AGCTGACCCA GCCGCTGCAG CAGGTGACGT CGTTGTTCAG 720

CCAGGTGGGC GGCACCGGCG GCGGCAACCC AGCCGACGAG GAAGCCGCGC AGATGGGCCT 780

GCTCGGCACC AGTCCGCTGT CGAACCATCC GCTGGCTGGT GGATCAGGCC CCAGCGCGGG 840

CGCGGGCCTG CTGCGCGCGG AGTCGCTACC TGGCGCAGGT GGGTCGTTGA CCCGCACGCC 900

GCTGATGTCT CAGCTGATCG AAAAGCCGGT TGCCCCCTCG GTGATGCCGG CGGCTGCTGC 960

CGGATCGTCG GCGACGGGTG GCGCCGCTCC GGTGGGTGCG GGAGCGATGG GCCAGGGTGC 1020

GCAATCCGGC GGCTCCACCA GGCCGGGTCT GGTCGCGCCG GCACCGCTCG CGCAGGAGCG 1080

TGAAGAAGAC GACGAGGACG ACTGGGACGA AGAGGACGAC TGGTGAGCTC CCGTAATGAC 1140

AACAGACTTC CCGGCCACCC GGGCCGGAAG ACTTGCCAAC ATTTTGGCGA GGAAGGTAAA 1200

GAGAGAAAGT AGTCCAGCAT GGCAGAGATG AAGACCGATG CCGCTACCCT CGCGCAGGAG 1260

GCAGGTAATT TCGAGCGGAT CTCCGGCGAC CTGAAAACCC AGATCGACCA GGTGGAGTCG 1320

ACGGCAGGTT CGTTGCAGGG CCAGTGGCGC GGCGCGGCGG GGACGGCCGC CCAGGCCGCG 1380

GTGGTGCGCT TCCAAGAAGC AGCCAATAAG CAGAAGCAGG AACTCGACGA GATCTCGACG 1440

AATATTCGTC AGGCCGGCGT CCAATACTCG AGGGCCGACG AGGAGCAGCA GCAGGCGCTG 1500

TCCTCGCAAA TGGGCTTCTG ACCCGCTAAT ACGAAAAGAA ACGGAGCAAA AACATGACAG 1560

AGCAGCAGTG GAATTTCGCG GGTATCGAGG CCGCGGCAAG CGCAATCCAG GGAAAT 1616

(2) INFORMATION FOR SEQ ID NO:113:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 432 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

CTAGTGGATG GGACCATGGC CATTTTCTGC AGTCTCACTG CCTTCTGTGT TGACATTTTG 60

GCACGCCGGC GGAAACGAAG CACTGGGGTC GAAGAACGGC TGCGCTGCCA TATCGTCCGG 120

AGCTTCCATA CCTTCGTGCG GCCGGAAGAG CTTGTCGTAG TCGGCCGCCA TGACAACCTC 180

TCAGAGTGCG CTCAAACGTA TAAACACGAG AAAGGGCGAG ACCGACGGAA GGTCGAACTC 240

GCCCGATCCC GTGTTTCGCT ATTCTACGCG AACTCGGCGT TGCCCTATGC GAACATCCCA 300

GTGACGTTGC CTTCGGTCGA AGCCATTGCC TGACCGGCTT CGCTGATCGT CCGCGCCAGG 360

TTCTGCAGCG CGTTGTTCAG CTCGGTAGCC GTGGCGTCCC ATTTTTGCTG GACACCCTGG 420

TACGCCTCCG AA 432

(2) INFORMATION FOR SEQ ID NO:114:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 368 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

Met Leu Trp His Ala Met Pro Pro Glu Xaa Asn Thr Ala Arg Leu Met
1 5 10 15

Ala Gly Ala Gly Pro Ala Pro Met Leu Ala Ala Ala Ala Gly Trp Gln
20 25 30

Thr Leu Ser Ala Ala Leu Asp Ala Gln Ala Val Glu Leu Thr Ala Arg
35 40 45

Leu Asn Ser Leu Gly Glu Ala Trp Thr Gly Gly Gly Ser Asp Lys Ala
50 55 60

Leu Ala Ala Ala Thr Pro Met Val Val Trp Leu Gln Thr Ala Ser Thr
65 70 75 80

Gln Ala Lys Thr Arg Ala Met Gln Ala Thr Ala Gln Ala Ala Ala Tyr
85 90 95

Thr Gln Ala Met Ala Thr Thr Pro Ser Leu Pro Glu Ile Ala Ala Asn
100 105 110

His Ile Thr Gln Ala Val Leu Thr Ala Thr Asn Phe Phe Gly Ile Asn
115 120 125

```
Thr Ile Pro Ile Ala Leu Thr Glu Met Asp Tyr Phe Ile Arg Met Trp
    130                 135                 140

Asn Gln Ala Ala Leu Ala Met Glu Val Tyr Gln Ala Glu Thr Ala Val
145                 150                 155                 160

Asn Thr Leu Phe Glu Lys Leu Glu Pro Met Ala Ser Ile Leu Asp Pro
                165                 170                 175

Gly Ala Ser Gln Ser Thr Thr Asn Pro Ile Phe Gly Met Pro Ser Pro
            180                 185                 190

Gly Ser Ser Thr Pro Val Gly Gln Leu Pro Pro Ala Ala Thr Gln Thr
        195                 200                 205

Leu Gly Gln Leu Gly Glu Met Ser Gly Pro Met Gln Gln Leu Thr Gln
    210                 215                 220

Pro Leu Gln Gln Val Thr Ser Leu Phe Ser Gln Val Gly Gly Thr Gly
225                 230                 235                 240

Gly Gly Asn Pro Ala Asp Glu Glu Ala Ala Gln Met Gly Leu Leu Gly
                245                 250                 255

Thr Ser Pro Leu Ser Asn His Pro Leu Ala Gly Gly Ser Gly Pro Ser
            260                 265                 270

Ala Gly Ala Gly Leu Leu Arg Ala Glu Ser Leu Pro Gly Ala Gly Gly
        275                 280                 285

Ser Leu Thr Arg Thr Pro Leu Met Ser Gln Leu Ile Glu Lys Pro Val
    290                 295                 300

Ala Pro Ser Val Met Pro Ala Ala Ala Ala Gly Ser Ser Ala Thr Gly
305                 310                 315                 320

Gly Ala Ala Pro Val Gly Ala Gly Ala Met Gly Gln Gly Ala Gln Ser
                325                 330                 335

Gly Gly Ser Thr Arg Pro Gly Leu Val Ala Pro Ala Pro Leu Ala Gln
            340                 345                 350

Glu Arg Glu Glu Asp Asp Glu Asp Asp Trp Asp Glu Glu Asp Asp Trp
        355                 360                 365
```

(2) INFORMATION FOR SEQ ID NO:115:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 100 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

```
Met Ala Glu Met Lys Thr Asp Ala Ala Thr Leu Ala Gln Glu Ala Gly
1               5                   10                  15
```

Asn Phe Glu Arg Ile Ser Gly Asp Leu Lys Thr Gln Ile Asp Gln Val
20 25 30

Glu Ser Thr Ala Gly Ser Leu Gln Gly Gln Trp Arg Gly Ala Ala Gly
35 40 45

Thr Ala Ala Gln Ala Ala Val Val Arg Phe Gln Glu Ala Ala Asn Lys
50 55 60

Gln Lys Gln Glu Leu Asp Glu Ile Ser Thr Asn Ile Arg Gln Ala Gly
65 70 75 80

Val Gln Tyr Ser Arg Ala Asp Glu Glu Gln Gln Gln Ala Leu Ser Ser
85 90 95

Gln Met Gly Phe
100

(2) INFORMATION FOR SEQ ID NO:116:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 396 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:

GATCTCCGGC GACCTGAAAA CCCAGATCGA CCAGGTGGAG TCGACGGCAG GTTCGTTGCA 60

GGGCCAGTGG CGCGGCGCGG CGGGGACGGC CGCCCAGGCC GCGGTGGTGC GCTTCCAAGA 120

AGCAGCCAAT AAGCAGAAGC AGGAACTCGA CGAGATCTCG ACGAATATTC GTCAGGCCGG 180

CGTCCAATAC TCGAGGGCCG ACGAGGAGCA GCAGCAGGCG CTGTCCTCGC AAATGGGCTT 240

CTGACCCGCT AATACGAAAA GAAACGGAGC AAAAACATGA CAGAGCAGCA GTGGAATTTC 300

GCGGGTATCG AGGCCGCGGC AAGCGCAATC CAGGGAAATG TCACGTCCAT TCATTCCCTC 360

CTTGACGAGG GGAAGCAGTC CCTGACCAAG CTCGCA 396

(2) INFORMATION FOR SEQ ID NO:117:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 80 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

Ile Ser Gly Asp Leu Lys Thr Gln Ile Asp Gln Val Glu Ser Thr Ala

1 5 10 15

Gly Ser Leu Gln Gly Gln Trp Arg Gly Ala Ala Gly Thr Ala Ala Gln
20 25 30

Ala Ala Val Val Arg Phe Gln Glu Ala Ala Asn Lys Gln Lys Gln Glu
35 40 45

Leu Asp Glu Ile Ser Thr Asn Ile Arg Gln Ala Gly Val Gln Tyr Ser
50 55 60

Arg Ala Asp Glu Glu Gln Gln Gln Ala Leu Ser Ser Gln Met Gly Phe
65 70 75 80

(2) INFORMATION FOR SEQ ID NO:118:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 387 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

GTGGATCCCG ATCCCGTGTT TCGCTATTCT ACGCGAACTC GGCGTTGCCC TATGCGAACA 60

TCCCAGTGAC GTTGCCTTCG GTCGAAGCCA TTGCCTGACC GGCTTCGCTG ATCGTCCGCG 120

CCAGGTTCTG CAGCGCGTTG TTCAGCTCGG TAGCCGTGGC GTCCCATTTT TGCTGGACAC 180

CCTGGTACGC CTCCGAACCG CTACCGCCCC AGGCCGCTGC GAGCTTGGTC AGGGACTGCT 240

TCCCCTCGTC AAGGAGGGAA TGAATGGACG TGACATTTCC CTGGATTGCG CTTGCCGCGG 300

CCTCGATACC CGCGAAATTC CACTGCTGCT CTGTCATGTT TTTGCTCCGT TTCTTTTCGT 360

ATTAGCGGGT CAGAAGCCCA TTTGCGA 387

(2) INFORMATION FOR SEQ ID NO:119:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 272 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

CGGCACGAGG ATCTCGGTTG GCCCAACGGC GCTGGCGAGG GCTCCGTTCC GGGGGCGAGC 60

TGCGCGCCGG ATGCTTCCTC TGCCCGCAGC CGCGCCTGGA TGGATGGACC AGTTGCTACC 120

TTCCCGACGT TTCGTTCGGT GTCTGTGCGA TAGCGGTGAC CCCGGCGCGC ACGTCGGGAG 180

TGTTGGGGGG CAGGCCGGGT CGGTGGTTCG GCCGGGGACG CAGACGGTCT GGACGGAACG 240

GGCGGGGGTT CGCCGATTGG CATCTTTGCC CA 272

(2) INFORMATION FOR SEQ ID NO:120:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

Asp Pro Val Asp Ala Val Ile Asn Thr Thr Cys Asn Tyr Gly Gln Val
1 5 10 15

Val Ala Ala Leu
20

(2) INFORMATION FOR SEQ ID NO:121:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

Ala Val Glu Ser Gly Met Leu Ala Leu Gly Thr Pro Ala Pro Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:122:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

Ala Ala Met Lys Pro Arg Thr Gly Asp Gly Pro Leu Glu Ala Ala Lys
1 5 10 15

Glu Gly Arg

GAGAGAATTC TCAGAAGCCC ATTTGCGAGG ACA 33

(2) INFORMATION FOR SEQ ID NO:152:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1993 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 152..1273

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:152:

```
TGTTCTTCGA CGGCAGGCTG GTGGAGGAAG GGCCCACCGA ACAGCTGTTC TCCTCGCCGA      60

AGCATGCGGA AACCGCCCGA TACGTCGCCG GACTGTCGGG GGACGTCAAG GACGCCAAGC     120

GCGGAAATTG AAGAGCACAG AAAGGTATGG C GTG AAA ATT CGT TTG CAT ACG         172
                                    Val Lys Ile Arg Leu His Thr
                                      1               5

CTG TTG GCC GTG TTG ACC GCT GCG CCG CTG CTG CTA GCA GCG GCG GGC         220
Leu Leu Ala Val Leu Thr Ala Ala Pro Leu Leu Leu Ala Ala Ala Gly
         10                  15                  20

TGT GGC TCG AAA CCA CCG AGC GGT TCG CCT GAA ACG GGC GCC GGC GCC         268
Cys Gly Ser Lys Pro Pro Ser Gly Ser Pro Glu Thr Gly Ala Gly Ala
     25                  30                  35

GGT ACT GTC GCG ACT ACC CCC GCG TCG TCG CCG GTG ACG TTG GCG GAG         316
Gly Thr Val Ala Thr Thr Pro Ala Ser Ser Pro Val Thr Leu Ala Glu
 40                  45                  50                  55

ACC GGT AGC ACG CTG CTC TAC CCG CTG TTC AAC CTG TGG GGT CCG GCC         364
Thr Gly Ser Thr Leu Leu Tyr Pro Leu Phe Asn Leu Trp Gly Pro Ala
                 60                  65                  70

TTT CAC GAG AGG TAT CCG AAC GTC ACG ATC ACC GCT CAG GGC ACC GGT         412
Phe His Glu Arg Tyr Pro Asn Val Thr Ile Thr Ala Gln Gly Thr Gly
             75                  80                  85

TCT GGT GCC GGG ATC GCG CAG GCC GCC GCC GGG ACG GTC AAC ATT GGG         460
Ser Gly Ala Gly Ile Ala Gln Ala Ala Ala Gly Thr Val Asn Ile Gly
         90                  95                 100

GCC TCC GAC GCC TAT CTG TCG GAA GGT GAT ATG GCC GCG CAC AAG GGG         508
Ala Ser Asp Ala Tyr Leu Ser Glu Gly Asp Met Ala Ala His Lys Gly
    105                 110                 115

CTG ATG AAC ATC GCG CTA GCC ATC TCC GCT CAG CAG GTC AAC TAC AAC         556
Leu Met Asn Ile Ala Leu Ala Ile Ser Ala Gln Gln Val Asn Tyr Asn
120                 125                 130                 135
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CTG CCC GGA GTG AGC GAG CAC CTC AAG CTG AAC GGA AAA GTC CTG GCG        604
Leu Pro Gly Val Ser Glu His Leu Lys Leu Asn Gly Lys Val Leu Ala
                140                 145                 150

GCC ATG TAC CAG GGC ACC ATC AAA ACC TGG GAC GAC CCG CAG ATC GCT        652
Ala Met Tyr Gln Gly Thr Ile Lys Thr Trp Asp Asp Pro Gln Ile Ala
            155                 160                 165

GCG CTC AAC CCC GGC GTG AAC CTG CCC GGC ACC GCG GTA GTT CCG CTG        700
Ala Leu Asn Pro Gly Val Asn Leu Pro Gly Thr Ala Val Val Pro Leu
        170                 175                 180

CAC CGC TCC GAC GGG TCC GGT GAC ACC TTC TTG TTC ACC CAG TAC CTG        748
His Arg Ser Asp Gly Ser Gly Asp Thr Phe Leu Phe Thr Gln Tyr Leu
    185                 190                 195

TCC AAG CAA GAT CCC GAG GGC TGG GGC AAG TCG CCC GGC TTC GGC ACC        796
Ser Lys Gln Asp Pro Glu Gly Trp Gly Lys Ser Pro Gly Phe Gly Thr
200                 205                 210                 215

ACC GTC GAC TTC CCG GCG GTG CCG GGT GCG CTG GGT GAG AAC GGC AAC        844
Thr Val Asp Phe Pro Ala Val Pro Gly Ala Leu Gly Glu Asn Gly Asn
                220                 225                 230

GGC GGC ATG GTG ACC GGT TGC GCC GAG ACA CCG GGC TGC GTG GCC TAT        892
Gly Gly Met Val Thr Gly Cys Ala Glu Thr Pro Gly Cys Val Ala Tyr
            235                 240                 245

ATC GGC ATC AGC TTC CTC GAC CAG GCC AGT CAA CGG GGA CTC GGC GAG        940
Ile Gly Ile Ser Phe Leu Asp Gln Ala Ser Gln Arg Gly Leu Gly Glu
        250                 255                 260

GCC CAA CTA GGC AAT AGC TCT GGC AAT TTC TTG TTG CCC GAC GCG CAA        988
Ala Gln Leu Gly Asn Ser Ser Gly Asn Phe Leu Leu Pro Asp Ala Gln
    265                 270                 275

AGC ATT CAG GCC GCG GCG GCT GGC TTC GCA TCG AAA ACC CCG GCG AAC       1036
Ser Ile Gln Ala Ala Ala Ala Gly Phe Ala Ser Lys Thr Pro Ala Asn
280                 285                 290                 295

CAG GCG ATT TCG ATG ATC GAC GGG CCC GCC CCG GAC GGC TAC CCG ATC       1084
Gln Ala Ile Ser Met Ile Asp Gly Pro Ala Pro Asp Gly Tyr Pro Ile
                300                 305                 310

ATC AAC TAC GAG TAC GCC ATC GTC AAC AAC CGG CAA AAG GAC GCC GCC       1132
Ile Asn Tyr Glu Tyr Ala Ile Val Asn Asn Arg Gln Lys Asp Ala Ala
            315                 320                 325

ACC GCG CAG ACC TTG CAG GCA TTT CTG CAC TGG GCG ATC ACC GAC GGC       1180
Thr Ala Gln Thr Leu Gln Ala Phe Leu His Trp Ala Ile Thr Asp Gly
        330                 335                 340

AAC AAG GCC TCG TTC CTC GAC CAG GTT CAT TTC CAG CCG CTG CCG CCC       1228
Asn Lys Ala Ser Phe Leu Asp Gln Val His Phe Gln Pro Leu Pro Pro
    345                 350                 355

GCG GTG GTG AAG TTG TCT GAC GCG TTG ATC GCG ACG ATT TCC AGC           1273
Ala Val Val Lys Leu Ser Asp Ala Leu Ile Ala Thr Ile Ser Ser
360                 365                 370

TAGCCTCGTT GACCACCACG CGACAGCAAC CTCCGTCGGG CCATCGGGCT GCTTTGCGGA     1333
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GCATGCTGGC CCGTGCCGGT GAAGTCGGCC GCGCTGGCCC GGCCATCCGG TGGTTGGGTG 1393

GGATAGGTGC GGTGATCCCG CTGCTTGCGC TGGTCTTGGT GCTGGTGGTG CTGGTCATCG 1453

AGGCGATGGG TGCGATCAGG CTCAACGGGT TGCATTTCTT CACCGCCACC GAATGGAATC 1513

CAGGCAACAC CTACGGCGAA ACCGTTGTCA CCGACGCGTC GCCCATCCGG TCGGCGCCTA 1573

CTACGGGGCG TTGCCGCTGA TCGTCGGGAC GCTGGCGACC TCGGCAATCG CCCTGATCAT 1633

CGCGGTGCCG GTCTCTGTAG GAGCGGCGCT GGTGATCGTG GAACGGCTGC CGAAACGGTT 1693

GGCCGAGGCT GTGGGAATAG TCCTGGAATT GCTCGCCGGA ATCCCCAGCG TGGTCGTCGG 1753

TTTGTGGGGG GCAATGACGT TCGGGCCGTT CATCGCTCAT CACATCGCTC CGGTGATCGC 1813

TCACAACGCT CCCGATGTGC CGGTGCTGAA CTACTTGCGC GGCGACCCGG GCAACGGGGA 1873

GGGCATGTTG GTGTCCGGTC TGGTGTTGGC GGTGATGGTC GTTCCCATTA TCGCCACCAC 1933

CACTCATGAC CTGTTCCGGC AGGTGCCGGT GTTGCCCCGG GAGGGCGCGA TCGGGAATTC 1993

(2) INFORMATION FOR SEQ ID NO:153:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 374 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:153:

```
Val Lys Ile Arg Leu His Thr Leu Leu Ala Val Leu Thr Ala Ala Pro
 1               5                  10                  15

Leu Leu Leu Ala Ala Ala Gly Cys Gly Ser Lys Pro Pro Ser Gly Ser
            20                  25                  30

Pro Glu Thr Gly Ala Gly Ala Gly Thr Val Ala Thr Thr Pro Ala Ser
        35                  40                  45

Ser Pro Val Thr Leu Ala Glu Thr Gly Ser Thr Leu Leu Tyr Pro Leu
    50                  55                  60

Phe Asn Leu Trp Gly Pro Ala Phe His Glu Arg Tyr Pro Asn Val Thr
65                  70                  75                  80

Ile Thr Ala Gln Gly Thr Gly Ser Gly Ala Gly Ile Ala Gln Ala Ala
                85                  90                  95

Ala Gly Thr Val Asn Ile Gly Ala Ser Asp Ala Tyr Leu Ser Glu Gly
            100                 105                 110

Asp Met Ala Ala His Lys Gly Leu Met Asn Ile Ala Leu Ala Ile Ser
        115                 120                 125

Ala Gln Gln Val Asn Tyr Asn Leu Pro Gly Val Ser Glu His Leu Lys
    130                 135                 140
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Leu Asn Gly Lys Val Leu Ala Ala Met Tyr Gln Gly Thr Ile Lys Thr
145                 150                 155                 160

Trp Asp Asp Pro Gln Ile Ala Ala Leu Asn Pro Gly Val Asn Leu Pro
                165                 170                 175

Gly Thr Ala Val Val Pro Leu His Arg Ser Asp Gly Ser Gly Asp Thr
            180                 185                 190

Phe Leu Phe Thr Gln Tyr Leu Ser Lys Gln Asp Pro Glu Gly Trp Gly
        195                 200                 205

Lys Ser Pro Gly Phe Gly Thr Thr Val Asp Phe Pro Ala Val Pro Gly
    210                 215                 220

Ala Leu Gly Glu Asn Gly Asn Gly Gly Met Val Thr Gly Cys Ala Glu
225                 230                 235                 240

Thr Pro Gly Cys Val Ala Tyr Ile Gly Ile Ser Phe Leu Asp Gln Ala
                245                 250                 255

Ser Gln Arg Gly Leu Gly Glu Ala Gln Leu Gly Asn Ser Ser Gly Asn
            260                 265                 270

Phe Leu Leu Pro Asp Ala Gln Ser Ile Gln Ala Ala Ala Ala Gly Phe
        275                 280                 285

Ala Ser Lys Thr Pro Ala Asn Gln Ala Ile Ser Met Ile Asp Gly Pro
    290                 295                 300

Ala Pro Asp Gly Tyr Pro Ile Ile Asn Tyr Glu Tyr Ala Ile Val Asn
305                 310                 315                 320

Asn Arg Gln Lys Asp Ala Ala Thr Ala Gln Thr Leu Gln Ala Phe Leu
                325                 330                 335

His Trp Ala Ile Thr Asp Gly Asn Lys Ala Ser Phe Leu Asp Gln Val
            340                 345                 350

His Phe Gln Pro Leu Pro Pro Ala Val Val Lys Leu Ser Asp Ala Leu
        355                 360                 365

Ile Ala Thr Ile Ser Ser
    370
```

(2) INFORMATION FOR SEQ ID NO:154:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1993 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:154:

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TGTTCTTCGA CGGCAGGCTG GTGGAGGAAG GGCCCACCGA ACAGCTGTTC TCCTCGCCGA      60
```

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:212:

CTTCATGGAA TTCTCAGGCC GGTAAGGTCC GCTGCGG 37

(2) INFORMATION FOR SEQ ID NO:213:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7676 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:213:

TGGCGAATGG GACGCGCCCT GTAGCGGCGC ATTAAGCGCG GCGGGTGTGG TGGTTACGCG 60

CAGCGTGACC GCTACACTTG CCAGCGCCCT AGCGCCCGCT CCTTTCGCTT TCTTCCCTTC 120

CTTTCTCGCC ACGTTCGCCG GCTTTCCCCG TCAAGCTCTA AATCGGGGGC TCCCTTTAGG 180

GTTCCGATTT AGTGCTTTAC GGCACCTCGA CCCCAAAAAA CTTGATTAGG GTGATGGTTC 240

ACGTAGTGGG CCATCGCCCT GATAGACGGT TTTTCGCCCT TTGACGTTGG AGTCCACGTT 300

CTTTAATAGT GGACTCTTGT TCCAAACTGG AACAACACTC AACCCTATCT CGGTCTATTC 360

TTTTGATTTA TAAGGGATTT TGCCGATTTC GGCCTATTGG TTAAAAAATG AGCTGATTTA 420

ACAAAAATTT AACGCGAATT TTAACAAAAT ATTAACGTTT ACAATTTCAG GTGGCACTTT 480

TCGGGGAAAT GTGCGCGGAA CCCCTATTTG TTTATTTTTC TAAATACATT CAAATATGTA 540

TCCGCTCATG AATTAATTCT TAGAAAAACT CATCGAGCAT CAAATGAAAC TGCAATTTAT 600

TCATATCAGG ATTATCAATA CCATATTTTT GAAAAAGCCG TTTCTGTAAT GAAGGAGAAA 660

ACTCACCGAG GCAGTTCCAT AGGATGGCAA GATCCTGGTA TCGGTCTGCG ATTCCGACTC 720

GTCCAACATC AATACAACCT ATTAATTTCC CCTCGTCAAA AATAAGGTTA TCAAGTGAGA 780

AATCACCATG AGTGACGACT GAATCCGGTG AGAATGGCAA AAGTTTATGC ATTTCTTTCC 840

AGACTTGTTC AACAGGCCAG CCATTACGCT CGTCATCAAA ATCACTCGCA TCAACCAAAC 900

CGTTATTCAT TCGTGATTGC GCCTGAGCGA GACGAAATAC GCGATCGCTG TTAAAAGGAC 960

AATTACAAAC AGGAATCGAA TGCAACCGGC GCAGGAACAC TGCCAGCGCA TCAACAATAT 1020

TTTCACCTGA ATCAGGATAT TCTTCTAATA CCTGGAATGC TGTTTTCCCG GGGATCGCAG 1080

TGGTGAGTAA CCATGCATCA TCAGGAGTAC GGATAAAATG CTTGATGGTC GGAAGAGGCA 1140

TAAATTCCGT CAGCCAGTTT AGTCTGACCA TCTCATCTGT AACATCATTG GCAACGCTAC 1200

CTTTGCCATG TTTCAGAAAC AACTCTGGCG CATCGGGCTT CCCATACAAT CGATAGATTG 1260

TCGCACCTGA TTGCCCGACA TTATCGCGAG CCCATTTATA CCCATATAAA TCAGCATCCA 1320

TGTTGGAATT TAATCGCGGC CTAGAGCAAG ACGTTTCCCG TTGAATATGG CTCATAACAC 1380

CCCTTGTATT ACTGTTTATG TAAGCAGACA GTTTTATTGT TCATGACCAA AATCCCTTAA 1440

CGTGAGTTTT CGTTCCACTG AGCGTCAGAC CCCGTAGAAA AGATCAAAGG ATCTTCTTGA 1500

GATCCTTTTT TTCTGCGCGT AATCTGCTGC TTGCAAACAA AAAAACCACC GCTACCAGCG 1560

GTGGTTTGTT TGCCGGATCA AGAGCTACCA ACTCTTTTTC CGAAGGTAAC TGGCTTCAGC 1620

AGAGCGCAGA TACCAAATAC TGTCCTTCTA GTGTAGCCGT AGTTAGGCCA CCACTTCAAG 1680

AACTCTGTAG CACCGCCTAC ATACCTCGCT CTGCTAATCC TGTTACCAGT GGCTGCTGCC 1740

AGTGGCGATA AGTCGTGTCT TACCGGGTTG GACTCAAGAC GATAGTTACC GGATAAGGCG 1800

CAGCGGTCGG GCTGAACGGG GGGTTCGTGC ACACAGCCCA GCTTGGAGCG AACGACCTAC 1860

ACCGAACTGA GATACCTACA GCGTGAGCTA TGAGAAAGCG CCACGCTTCC CGAAGGGAGA 1920

AAGGCGGACA GGTATCCGGT AAGCGGCAGG GTCGGAACAG GAGAGCGCAC GAGGGAGCTT 1980

CCAGGGGGAA ACGCCTGGTA TCTTTATAGT CCTGTCGGGT TTCGCCACCT CTGACTTGAG 2040

CGTCGATTTT TGTGATGCTC GTCAGGGGGG CGGAGCCTAT GGAAAAACGC CAGCAACGCG 2100

GCCTTTTTAC GGTTCCTGGC CTTTTGCTGG CCTTTTGCTC ACATGTTCTT TCCTGCGTTA 2160

TCCCCTGATT CTGTGGATAA CCGTATTACC GCCTTTGAGT GAGCTGATAC CGCTCGCCGC 2220

AGCCGAACGA CCGAGCGCAG CGAGTCAGTG AGCGAGGAAG CGGAAGAGCG CCTGATGCGG 2280

TATTTTCTCC TTACGCATCT GTGCGGTATT TCACACCGCA TATATGGTGC ACTCTCAGTA 2340

CAATCTGCTC TGATGCCGCA TAGTTAAGCC AGTATACACT CCGCTATCGC TACGTGACTG 2400

GGTCATGGCT GCGCCCCGAC ACCCGCCAAC ACCCGCTGAC GCGCCCTGAC GGGCTTGTCT 2460

GCTCCCGGCA TCCGCTTACA GACAAGCTGT GACCGTCTCC GGGAGCTGCA TGTGTCAGAG 2520

GTTTTCACCG TCATCACCGA AACGCGCGAG GCAGCTGCGG TAAAGCTCAT CAGCGTGGTC 2580

GTGAAGCGAT TCACAGATGT CTGCCTGTTC ATCCGCGTCC AGCTCGTTGA GTTTCTCCAG 2640

AAGCGTTAAT GTCTGGCTTC TGATAAAGCG GGCCATGTTA AGGGCGGTTT TTTCCTGTTT 2700

GGTCACTGAT GCCTCCGTGT AAGGGGGATT TCTGTTCATG GGGGTAATGA TACCGATGAA 2760

ACGAGAGAGG ATGCTCACGA TACGGGTTAC TGATGATGAA CATGCCCGGT TACTGGAACG 2820

TTGTGAGGGT AAACAACTGG CGGTATGGAT GCGGCGGGAC CAGAGAAAAA TCACTCAGGG 2880

TCAATGCCAG CGCTTCGTTA ATACAGATGT AGGTGTTCCA CAGGGTAGCC AGCAGCATCC 2940

TGCGATGCAG ATCCGGAACA TAATGGTGCA GGGCGCTGAC TTCCGCGTTT CCAGACTTTA 3000

CGAAACACGG AAACCGAAGA CCATTCATGT TGTTGCTCAG GTCGCAGACG TTTTGCAGCA 3060

GCAGTCGCTT CACGTTCGCT CGCGTATCGG TGATTCATTC TGCTAACCAG TAAGGCAACC 3120

CCGCCAGCCT AGCCGGGTCC TCAACGACAG GAGCACGATC ATGCGCACCC GTGGGGCCGC 3180

CATGCCGGCG ATAATGGCCT GCTTCTCGCC GAAACGTTTG GTGGCGGGAC CAGTGACGAA 3240

GGCTTGAGCG AGGGCGTGCA AGATTCCGAA TACCGCAAGC GACAGGCCGA TCATCGTCGC 3300

GCTCCAGCGA AAGCGGTCCT CGCCGAAAAT GACCCAGAGC GCTGCCGGCA CCTGTCCTAC 3360

GAGTTGCATG ATAAAGAAGA CAGTCATAAG TGCGGCGACG ATAGTCATGC CCCGCGCCCA 3420

CCGGAAGGAG CTGACTGGGT TGAAGGCTCT CAAGGGCATC GGTCGAGATC CCGGTGCCTA 3480

ATGAGTGAGC TAACTTACAT TAATTGCGTT GCGCTCACTG CCCGCTTTCC AGTCGGGAAA 3540

CCTGTCGTGC CAGCTGCATT AATGAATCGG CCAACGCGCG GGGAGAGGCG GTTTGCGTAT 3600

TGGGCGCCAG GGTGGTTTTT CTTTTCACCA GTGAGACGGG CAACAGCTGA TTGCCCTTCA 3660

CCGCCTGGCC CTGAGAGAGT TGCAGCAAGC GGTCCACGCT GGTTTGCCCC AGCAGGCGAA 3720

AATCCTGTTT GATGGTGGTT AACGGCGGGA TATAACATGA GCTGTCTTCG GTATCGTCGT 3780

ATCCCACTAC CGAGATATCC GCACCAACGC GCAGCCCGGA CTCGGTAATG GCGCGCATTG 3840

CGCCCAGCGC CATCTGATCG TTGGCAACCA GCATCGCAGT GGGAACGATG CCCTCATTCA 3900

GCATTTGCAT GGTTTGTTGA AAACCGGACA TGGCACTCCA GTCGCCTTCC CGTTCCGCTA 3960

TCGGCTGAAT TTGATTGCGA GTGAGATATT TATGCCAGCC AGCCAGACGC AGACGCGCCG 4020

AGACAGAACT TAATGGGCCC GCTAACAGCG CGATTTGCTG GTGACCCAAT GCGACCAGAT 4080

GCTCCACGCC CAGTCGCGTA CCGTCTTCAT GGGAGAAAAT AATACTGTTG ATGGGTGTCT 4140

GGTCAGAGAC ATCAAGAAAT AACGCCGGAA CATTAGTGCA GGCAGCTTCC ACAGCAATGG 4200

CATCCTGGTC ATCCAGCGGA TAGTTAATGA TCAGCCCACT GACGCGTTGC GCGAGAAGAT 4260

TGTGCACCGC CGCTTTACAG GCTTCGACGC CGCTTCGTTC TACCATCGAC ACCACCACGC 4320

TGGCACCCAG TTGATCGGCG CGAGATTTAA TCGCCGCGAC AATTTGCGAC GGCGCGTGCA 4380

GGGCCAGACT GGAGGTGGCA ACGCCAATCA GCAACGACTG TTTGCCCGCC AGTTGTTGTG 4440

CCACGCGGTT GGGAATGTAA TTCAGCTCCG CCATCGCCGC TTCCACTTTT TCCCGCGTTT 4500

TCGCAGAAAC GTGGCTGGCC TGGTTCACCA CGCGGGAAAC GGTCTGATAA GAGACACCGG 4560

CATACTCTGC GACATCGTAT AACGTTACTG GTTTCACATT CACCACCCTG AATTGACTCT 4620

CTTCCGGGCG CTATCATGCC ATACCGCGAA AGGTTTTGCG CCATTCGATG GTGTCCGGGA 4680

TCTCGACGCT CTCCCTTATG CGACTCCTGC ATTAGGAAGC AGCCCAGTAG TAGGTTGAGG 4740

CCGTTGAGCA CCGCCGCCGC AAGGAATGGT GCATGCAAGG AGATGGCGCC CAACAGTCCC 4800

CCGGCCACGG GGCCTGCCAC CATACCCACG CCGAAACAAG CGCTCATGAG CCCGAAGTGG 4860

CGAGCCCGAT CTTCCCCATC GGTGATGTCG GCGATATAGG CGCCAGCAAC CGCACCTGTG 4920

GCGCCGGTGA TGCCGGCCAC GATGCGTCCG GCGTAGAGGA TCGAGATCTC GATCCCGCGA 4980

AATTAATACG ACTCACTATA GGGGAATTGT GAGCGGATAA CAATTCCCCT CTAGAAATAA 5040

TTTTGTTTAA CTTTAAGAAG GAGATATACA TATGGGCCAT CATCATCATC ATCACGTGAT 5100

CGACATCATC GGGACCAGCC CCACATCCTG GGAACAGGCG GCGGCGGAGG CGGTCCAGCG 5160

GGCGCGGGAT AGCGTCGATG ACATCCGCGT CGCTCGGGTC ATTGAGCAGG ACATGGCCGT 5220

GGACAGCGCC GGCAAGATCA CCTACCGCAT CAAGCTCGAA GTGTCGTTCA AGATGAGGCC 5280

GGCGCAACCG AGGGGCTCGA AACCACCGAG CGGTTCGCCT GAAACGGGCG CCGGCGCCGG 5340

TACTGTCGCG ACTACCCCCG CGTCGTCGCC GGTGACGTTG GCGGAGACCG GTAGCACGCT 5400

GCTCTACCCG CTGTTCAACC TGTGGGGTCC GGCCTTTCAC GAGAGGTATC CGAACGTCAC 5460

GATCACCGCT CAGGGCACCG GTTCTGGTGC CGGGATCGCG CAGGCCGCCG CCGGGACGGT 5520

CAACATTGGG GCCTCCGACG CCTATCTGTC GGAAGGTGAT ATGGCCGCGC ACAAGGGGCT 5580

GATGAACATC GCGCTAGCCA TCTCCGCTCA GCAGGTCAAC TACAACCTGC CCGGAGTGAG 5640

CGAGCACCTC AAGCTGAACG GAAAAGTCCT GGCGGCCATG TACCAGGGCA CCATCAAAAC 5700

CTGGGACGAC CCGCAGATCG CTGCGCTCAA CCCCGGCGTG AACCTGCCCG GCACCGCGGT 5760

AGTTCCGCTG CACCGCTCCG ACGGGTCCGG TGACACCTTC TTGTTCACCC AGTACCTGTC 5820

CAAGCAAGAT CCCGAGGGCT GGGGCAAGTC GCCCGGCTTC GGCACCACCG TCGACTTCCC 5880

GGCGGTGCCG GGTGCGCTGG GTGAGAACGG CAACGGCGGC ATGGTGACCG GTTGCGCCGA 5940

GACACCGGGC TGCGTGGCCT ATATCGGCAT CAGCTTCCTC GACCAGGCCA GTCAACGGGG 6000

ACTCGGCGAG GCCCAACTAG GCAATAGCTC TGGCAATTTC TTGTTGCCCG ACGCGCAAAG 6060

CATTCAGGCC GCGGCGGCTG GCTTCGCATC GAAAACCCCG GCGAACCAGG CGATTTCGAT 6120

GATCGACGGG CCCGCCCCGG ACGGCTACCC GATCATCAAC TACGAGTACG CCATCGTCAA 6180

CAACCGGCAA AAGGACGCCG CCACCGCGCA GACCTTGCAG GCATTTCTGC ACTGGGCGAT 6240

CACCGACGGC AACAAGGCCT CGTTCCTCGA CCAGGTTCAT TTCCAGCCGC TGCCGCCCGC 6300

GGTGGTGAAG TTGTCTGACG CGTTGATCGC GACGATTTCC AGCGCTGAGA TGAAGACCGA 6360

TGCCGCTACC CTCGCGCAGG AGGCAGGTAA TTTCGAGCGG ATCTCCGGCG ACCTGAAAAC 6420

CCAGATCGAC CAGGTGGAGT CGACGGCAGG TTCGTTGCAG GGCCAGTGGC GCGGCGCGGC 6480

GGGGACGGCC GCCCAGGCCG CGGTGGTGCG CTTCCAAGAA GCAGCCAATA AGCAGAAGCA 6540

GGAACTCGAC GAGATCTCGA CGAATATTCG TCAGGCCGGC GTCCAATACT CGAGGGCCGA 6600

CGAGGAGCAG CAGCAGGCGC TGTCCTCGCA AATGGGCTTT GTGCCCACAA CGGCCGCCTC 6660

GCCGCCGTCG ACCGCTGCAG CGCCACCCGC ACCGGCGACA CCTGTTGCCC CCCCACCACC 6720

GGCCGCCGCC AACACGCCGA ATGCCCAGCC GGGCGATCCC AACGCAGCAC CTCCGCCGGC 6780

CGACCCGAAC GCACCGCCGC CACCTGTCAT TGCCCCAAAC GCACCCCAAC CTGTCCGGAT 6840

CGACAACCCG GTTGGAGGAT TCAGCTTCGC GCTGCCTGCT GGCTGGGTGG AGTCTGACGC 6900

CGCCCACTTC GACTACGGTT CAGCACTCCT CAGCAAAACC ACCGGGGACC CGCCATTTCC 6960

CGGACAGCCG CCGCCGGTGG CCAATGACAC CCGTATCGTG CTCGGCCGGC TAGACCAAAA 7020

GCTTTACGCC AGCGCCGAAG CCACCGACTC CAAGGCCGCG GCCCGGTTGG GCTCGGACAT 7080

GGGTGAGTTC TATATGCCCT ACCCGGGCAC CCGGATCAAC CAGGAAACCG TCTCGCTTGA 7140

CGCCAACGGG GTGTCTGGAA GCGCGTCGTA TTACGAAGTC AAGTTCAGCG ATCCGAGTAA 7200

GCCGAACGGC CAGATCTGGA CGGGCGTAAT CGGCTCGCCC GCGGCGAACG CACCGGACGC 7260

CGGGCCCCCT CAGCGCTGGT TTGTGGTATG GCTCGGGACC GCCAACAACC CGGTGGACAA 7320

GGGCGCGGCC AAGGCGCTGG CCGAATCGAT CCGGCCTTTG GTCGCCCCGC CGCCGGCGCC 7380

GGCACCGGCT CCTGCAGAGC CCGCTCCGGC GCCGGCGCCG GCCGGGGAAG TCGCTCCTAC 7440

CCCGACGACA CCGACACCGC AGCGGACCTT ACCGGCCTGA GAATTCTGCA GATATCCATC 7500

ACACTGGCGG CCGCTCGAGC ACCACCACCA CCACCACTGA GATCCGGCTG CTAACAAAGC 7560

CCGAAAGGAA GCTGAGTTGG CTGCTGCCAC CGCTGAGCAA TAACTAGCAT AACCCCTTGG 7620

GGCCTCTAAA CGGGTCTTGA GGGGTTTTTT GCTGAAAGGA GGAACTATAT CCGGAT 7676

(2) INFORMATION FOR SEQ ID NO:214:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 802 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:214:

Met Gly His His His His His His Val Ile Asp Ile Ile Gly Thr Ser
1 5 10 15

Pro Thr Ser Trp Glu Gln Ala Ala Ala Glu Ala Val Gln Arg Ala Arg
20 25 30

Asp Ser Val Asp Asp Ile Arg Val Ala Arg Val Ile Glu Gln Asp Met
35 40 45

Ala Val Asp Ser Ala Gly Lys Ile Thr Tyr Arg Ile Lys Leu Glu Val
50 55 60

Ser Phe Lys Met Arg Pro Ala Gln Pro Arg Gly Ser Lys Pro Pro Ser
65 70 75 80

Gly Ser Pro Glu Thr Gly Ala Gly Ala Gly Thr Val Ala Thr Thr Pro
85 90 95

Ala Ser Ser Pro Val Thr Leu Ala Glu Thr Gly Ser Thr Leu Leu Tyr
100 105 110

Pro Leu Phe Asn Leu Trp Gly Pro Ala Phe His Glu Arg Tyr Pro Asn
115 120 125

Val Thr Ile Thr Ala Gln Gly Thr Gly Ser Gly Ala Gly Ile Ala Gln
130 135 140

Ala Ala Ala Gly Thr Val Asn Ile Gly Ala Ser Asp Ala Tyr Leu Ser
145 150 155 160

Glu Gly Asp Met Ala Ala His Lys Gly Leu Met Asn Ile Ala Leu Ala
165 170 175

Ile Ser Ala Gln Gln Val Asn Tyr Asn Leu Pro Gly Val Ser Glu His
180 185 190

Leu Lys Leu Asn Gly Lys Val Leu Ala Ala Met Tyr Gln Gly Thr Ile
195 200 205

Lys Thr Trp Asp Asp Pro Gln Ile Ala Ala Leu Asn Pro Gly Val Asn
210 215 220

Leu Pro Gly Thr Ala Val Val Pro Leu His Arg Ser Asp Gly Ser Gly
225 230 235 240

Asp Thr Phe Leu Phe Thr Gln Tyr Leu Ser Lys Gln Asp Pro Glu Gly
245 250 255

Trp Gly Lys Ser Pro Gly Phe Gly Thr Thr Val Asp Phe Pro Ala Val
260 265 270

Pro Gly Ala Leu Gly Glu Asn Gly Asn Gly Gly Met Val Thr Gly Cys
275 280 285

Ala Glu Thr Pro Gly Cys Val Ala Tyr Ile Gly Ile Ser Phe Leu Asp
290 295 300

Gln Ala Ser Gln Arg Gly Leu Gly Glu Ala Gln Leu Gly Asn Ser Ser
305 310 315 320

Gly Asn Phe Leu Leu Pro Asp Ala Gln Ser Ile Gln Ala Ala Ala Ala
325 330 335

Gly Phe Ala Ser Lys Thr Pro Ala Asn Gln Ala Ile Ser Met Ile Asp
340 345 350

Gly Pro Ala Pro Asp Gly Tyr Pro Ile Ile Asn Tyr Glu Tyr Ala Ile
355 360 365

Val Asn Asn Arg Gln Lys Asp Ala Ala Thr Ala Gln Thr Leu Gln Ala
370 375 380

Phe Leu His Trp Ala Ile Thr Asp Gly Asn Lys Ala Ser Phe Leu Asp
385 390 395 400

Gln Val His Phe Gln Pro Leu Pro Pro Ala Val Val Lys Leu Ser Asp
405 410 415

Ala Leu Ile Ala Thr Ile Ser Ser Ala Glu Met Lys Thr Asp Ala Ala
420 425 430

Thr Leu Ala Gln Glu Ala Gly Asn Phe Glu Arg Ile Ser Gly Asp Leu
435 440 445

Lys Thr Gln Ile Asp Gln Val Glu Ser Thr Ala Gly Ser Leu Gln Gly
450 455 460

Gln Trp Arg Gly Ala Ala Gly Thr Ala Ala Gln Ala Ala Val Val Arg
465 470 475 480

Phe Gln Glu Ala Ala Asn Lys Gln Lys Gln Glu Leu Asp Glu Ile Ser
485 490 495

Thr Asn Ile Arg Gln Ala Gly Val Gln Tyr Ser Arg Ala Asp Glu Glu
500 505 510

Gln Gln Gln Ala Leu Ser Ser Gln Met Gly Phe Val Pro Thr Thr Ala
515 520 525

Ala Ser Pro Pro Ser Thr Ala Ala Ala Pro Pro Ala Pro Ala Thr Pro
530 535 540

Val Ala Pro Pro Pro Pro Ala Ala Ala Asn Thr Pro Asn Ala Gln Pro
545 550 555 560

Gly Asp Pro Asn Ala Ala Pro Pro Pro Ala Asp Pro Asn Ala Pro Pro
565 570 575

Pro Pro Val Ile Ala Pro Asn Ala Pro Gln Pro Val Arg Ile Asp Asn
580 585 590

Pro Val Gly Gly Phe Ser Phe Ala Leu Pro Ala Gly Trp Val Glu Ser
595 600 605

Asp Ala Ala His Phe Asp Tyr Gly Ser Ala Leu Leu Ser Lys Thr Thr
610 615 620

Gly Asp Pro Pro Phe Pro Gly Gln Pro Pro Pro Val Ala Asn Asp Thr
625 630 635 640

Arg Ile Val Leu Gly Arg Leu Asp Gln Lys Leu Tyr Ala Ser Ala Glu
645 650 655

Ala Thr Asp Ser Lys Ala Ala Ala Arg Leu Gly Ser Asp Met Gly Glu
660 665 670

Phe Tyr Met Pro Tyr Pro Gly Thr Arg Ile Asn Gln Glu Thr Val Ser
675 680 685

Leu Asp Ala Asn Gly Val Ser Gly Ser Ala Ser Tyr Tyr Glu Val Lys
690 695 700

Phe Ser Asp Pro Ser Lys Pro Asn Gly Gln Ile Trp Thr Gly Val Ile
705 710 715 720

Gly Ser Pro Ala Ala Asn Ala Pro Asp Ala Gly Pro Pro Gln Arg Trp
725 730 735

Phe Val Val Trp Leu Gly Thr Ala Asn Asn Pro Val Asp Lys Gly Ala

740 745 750

Ala Lys Ala Leu Ala Glu Ser Ile Arg Pro Leu Val Ala Pro Pro Pro
755 760 765

Ala Pro Ala Pro Ala Pro Ala Glu Pro Ala Pro Ala Pro Ala Pro Ala
770 775 780

Gly Glu Val Ala Pro Thr Pro Thr Thr Pro Thr Pro Gln Arg Thr Leu
785 790 795 800

Pro Ala

CLAIMS

1. A polypeptide comprising an immunogenic portion of a soluble *M. tuberculosis* antigen, or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen has an N-terminal sequence selected from the group consisting of:

(a) Asp-Pro-Val-Asp-Ala-Val-Ile-Asn-Thr-Thr-Cys-Asn-Tyr-Gly-Gln-Val-Val-Ala-Ala-Leu; (SEQ ID No. 120)

(b) Ala-Val-Glu-Ser-Gly-Met-Leu-Ala-Leu-Gly-Thr-Pro-Ala-Pro-Ser; (SEQ ID No. 121)

(c) Ala-Ala-Met-Lys-Pro-Arg-Thr-Gly-Asp-Gly-Pro-Leu-Glu-Ala-Ala-Lys-Glu-Gly-Arg; (SEQ ID No. 122)

(d) Tyr-Tyr-Trp-Cys-Pro-Gly-Gln-Pro-Phe-Asp-Pro-Ala-Trp-Gly-Pro; (SEQ ID No. 123)

(e) Asp-Ile-Gly-Ser-Glu-Ser-Thr-Glu-Asp-Gln-Gln-Xaa-Ala-Val; (SEQ ID No. 124)

(f) Ala-Glu-Glu-Ser-Ile-Ser-Thr-Xaa-Glu-Xaa-Ile-Val-Pro; (SEQ ID No. 125)

(g) Asp-Pro-Glu-Pro-Ala-Pro-Pro-Val-Pro-Thr-Thr-Ala-Ala-Ser-Pro-Pro-Ser; (SEQ ID No. 126)

(h) Ala-Pro-Lys-Thr-Tyr-Xaa-Glu-Glu-Leu-Lys-Gly-Thr-Asp-Thr-Gly; (SEQ ID No. 127)

(i) Asp-Pro-Ala-Ser-Ala-Pro-Asp-Val-Pro-Thr-Ala-Ala-Gln-Leu-Thr-Ser-Leu-Leu-Asn-Ser-Leu-Ala-Asp-Pro-Asn-Val-Ser-Phe-Ala-Asn; (SEQ ID No. 128) and

(j) Ala-Pro-Glu-Ser-Gly-Ala-Gly-Leu-Gly-Gly-Thr-Val-Gln-Ala-Gly; (SEQ ID No. 136)

wherein Xaa may be any amino acid.

2. A polypeptide comprising an immunogenic portion of an *M. tuberculosis* antigen, or a variant of said antigen that differs only in conservative

substitutions and/or modifications, wherein said antigen has an N-terminal sequence selected from the group consisting of:

(a) Asp-Pro-Pro-Asp-Pro-His-Gln-Xaa-Asp-Met-Thr-Lys-Gly-Tyr-Tyr-Pro-Gly-Gly-Arg-Arg-Xaa-Phe; (SEQ ID No. 129) and

(b) Xaa-Tyr-Ile-Ala-Tyr-Xaa-Thr-Thr-Ala-Gly-Ile-Val-Pro-Gly-Lys-Ile-Asn-Val-His-Leu-Val; (SEQ ID No. 137), wherein Xaa may be any amino acid.

3. A polypeptide comprising an immunogenic portion of a soluble *M. tuberculosis* antigen, or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen comprises an amino acid sequence encoded by a DNA sequence selected from the group consisting of the sequences recited in SEQ ID Nos.: 1, 2, 4-10, 13-25, 52, 99 and 101, the complements of said sequences, and DNA sequences that hybridize to a sequence recited in SEQ ID Nos.: 1, 2, 4-10, 13-25, 52, 99 and 101 or a complement thereof under moderately stringent conditions.

4. A polypeptide comprising an immunogenic portion of a *M. tuberculosis* antigen, or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen comprises an amino acid sequence encoded by a DNA sequence selected from the group consisting of the sequences recited in SEQ ID Nos.: 26-51, 138, 139, 163-183 and 201, the complements of said sequences, and DNA sequences that hybridize to a sequence recited in SEQ ID Nos.: 26-51, 138, 139, 163-183 and 201 or a complement thereof under moderately stringent conditions.

5. A DNA molecule comprising a nucleotide sequence encoding a polypeptide according to any one of claims 1-4.

6. An expression vector comprising a DNA molecule according to claim 5.

7. A host cell transformed with an expression vector according to claim 6.

8. The host cell of claim 7 wherein the host cell is selected from the group consisting of *E. coli*, yeast and mammalian cells.

9. A pharmaceutical composition comprising one or more polypeptides according to any one of claims 1-4 and a physiologically acceptable carrier.

10. A pharmaceutical composition comprising one or more DNA molecules according to claim 5 and a physiologically acceptable carrier.

11. A pharmaceutical composition comprising one or more DNA sequences recited in SEQ ID Nos.: 3, 11, 12, 140 and 141; and a physiologically acceptable carrier.

12. A vaccine comprising one or more polypeptides according to any one of claims 1-4 and a non-specific immune response enhancer.

13. A vaccine comprising:

a polypeptide having an N-terminal sequence selected from the group consisting of sequences recited in SEQ ID NO: 134 and 135; and

a non-specific immune response enhancer.

14. A vaccine comprising:

one or more polypeptides encoded by a DNA sequence selected from the group consisting of SEQ ID Nos.: 3, 11, 12, 140 and 141, the complements of said sequences, and DNA sequences that hybridize to a sequence recited in SEQ ID Nos.: 3, 11, 12, 140 and 141; and

a non-specific immune response enhancer.

15. The vaccine of claims 12-14 wherein the non-specific immune response enhancer is an adjuvant.

16. A vaccine comprising one or more DNA molecules according to claim 5 and a non-specific immune response enhancer.

17. A vaccine comprising one or more DNA sequences recited in SEQ ID Nos.: 3, 11, 12, 140 and 141; and a non-specific immune response enhancer.

18. The vaccine of claims 16 or 17 wherein the non-specific immune response enhancer is an adjuvant.

19. A pharmaceutical composition according to any one of claims 9-11, for use in the manufacture of a medicament for inducing protective immunity in a patient.

20. A vaccine according to any one of claims 12-18, for use in the manufacture of a medicament for inducing protective immunity in a patient.

21. A fusion protein comprising two or more polypeptides according to any one of claims 1-4.

22. A fusion protein comprising one or more polypeptides according to any one of claims 1-4 and ESAT-6.

23. A fusion protein comprising one or more polypeptides according to any one of claims 1-4 and the *M. tuberculosis* antigen 38 kD (SEQ ID NO:155).

24. A pharmaceutical composition comprising a fusion protein according to any one of claims 21-23 and a physiologically acceptable carrier.

25. A vaccine comprising a fusion protein according to any one of claims 21-23 and a non-specific immune response enhancer.

26. The vaccine of claim 25 wherein the non-specific immune response enhancer is an adjuvant.

27. A pharmaceutical composition according to claim 24, for use in the manufacture of a medicament for inducing protective immunity in a patient.

28. A vaccine according to claims 25 or 26, for use in the manufcture of a medicament for inducing protective immunity in a patient.

29. A method for detecting tuberculosis in a patient, comprising:

(a) contacting dermal cells of a patient with one or more polypeptides according to any one of claims 1-4; and

(b) detecting an immune response on the patient's skin and therefrom detecting tuberculosis in the patient.

30. A method for detecting tuberculosis in a patient, comprising:

(a) contacting dermal cells of a patient with a polypeptide having an N-terminal sequence selected from the group consisting of sequences recited in SEQ ID NO: 134 and 135; and

(b) detecting an immune response on the patient's skin and therefrom detecting tuberculosis in the patient.

31. A method for detecting tuberculosis in a patient, comprising:

(a) contacting dermal cells of a patient with one or more polypeptides encoded by a DNA sequence selected from the group consisting of SEQ ID Nos.: 3, 11, 12, 140, 141, 156-160, 189-193, 199, 200 and 203, the complements of said sequences, and DNA sequences that hybridize to a sequence recited in SEQ ID Nos.: 3, 11, 12, 140, 141, 156-160, 189-193, 199, 200 and 203; and

(b) detecting an immune response on the patient's skin and therefrom detecting tuberculosis in the patient.

32. The method of any one of claims 29-31 wherein the immune response is induration.

33. A diagnostic kit comprising:

(a) a polypeptide according to any one of claims 1-4; and

(b) apparatus sufficient to contact said polypeptide with the dermal cells of a patient.

34. A diagnostic kit comprising:

(a) a polypeptide having an N-terminal sequence selected from the group consisting of sequences recited in SEQ ID NO: 134 and 135; and

(b) apparatus sufficient to contact said polypeptide with the dermal cells of a patient.

35. A diagnostic kit comprising:

(a) a polypeptide encoded by a DNA sequence selected from the group consisting of SEQ ID Nos.: 3, 11, 12, 140, 141, 156-160, 189-193, 199, 200 and 203, the complements of said sequences, and DNA sequences that hybridize to a sequence recited in SEQ ID Nos.: 3, 11, 12, 140, 141, 156-160, 189-193, 199, 200 and 203; and

(b) apparatus sufficient to contact said polypeptide with the dermal cells of a patient.

36. A diagnostic kit comprising:

(a) a fusion protein according to any one of claims 21-23; and

(b) apparatus sufficient to contact said fusion protein with the dermal cells of a patient.

37. A fusion protein according to claim 23 comprising an amino acid sequence selected from the group consisting of sequences recited in SEQ ID NO: 153 and 209.

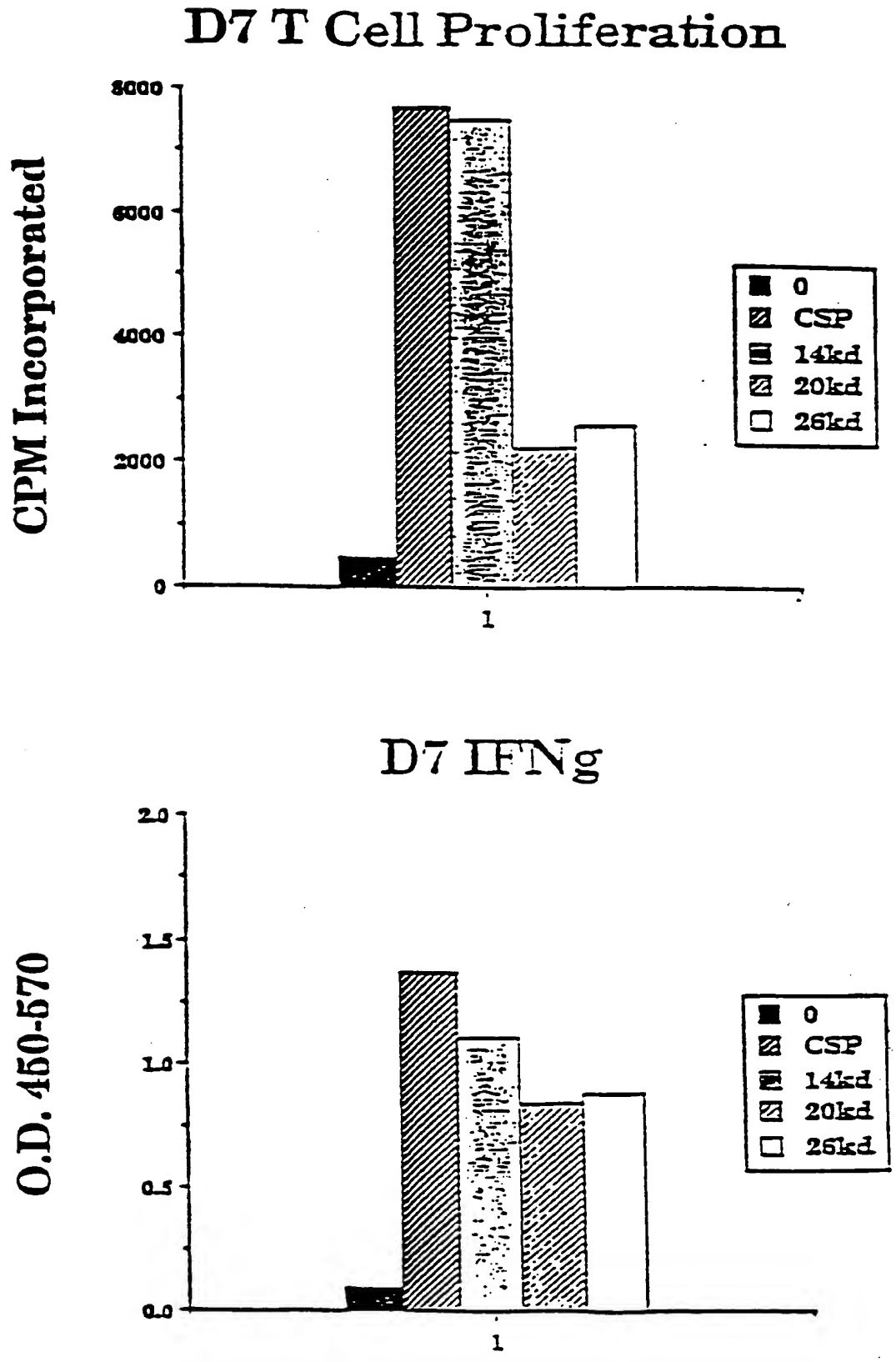

D7 T Cell Proliferation
CPM Incorporated
8000
6000
4000
2000
0
1
0
CSP
14kd
20kd
26kd
D7 IFNg
O.D. 450-570
2.0
1.5
1.0
0.5
0.0
1
0
CSP
14kd
20kd
26kd

FIG. 1A

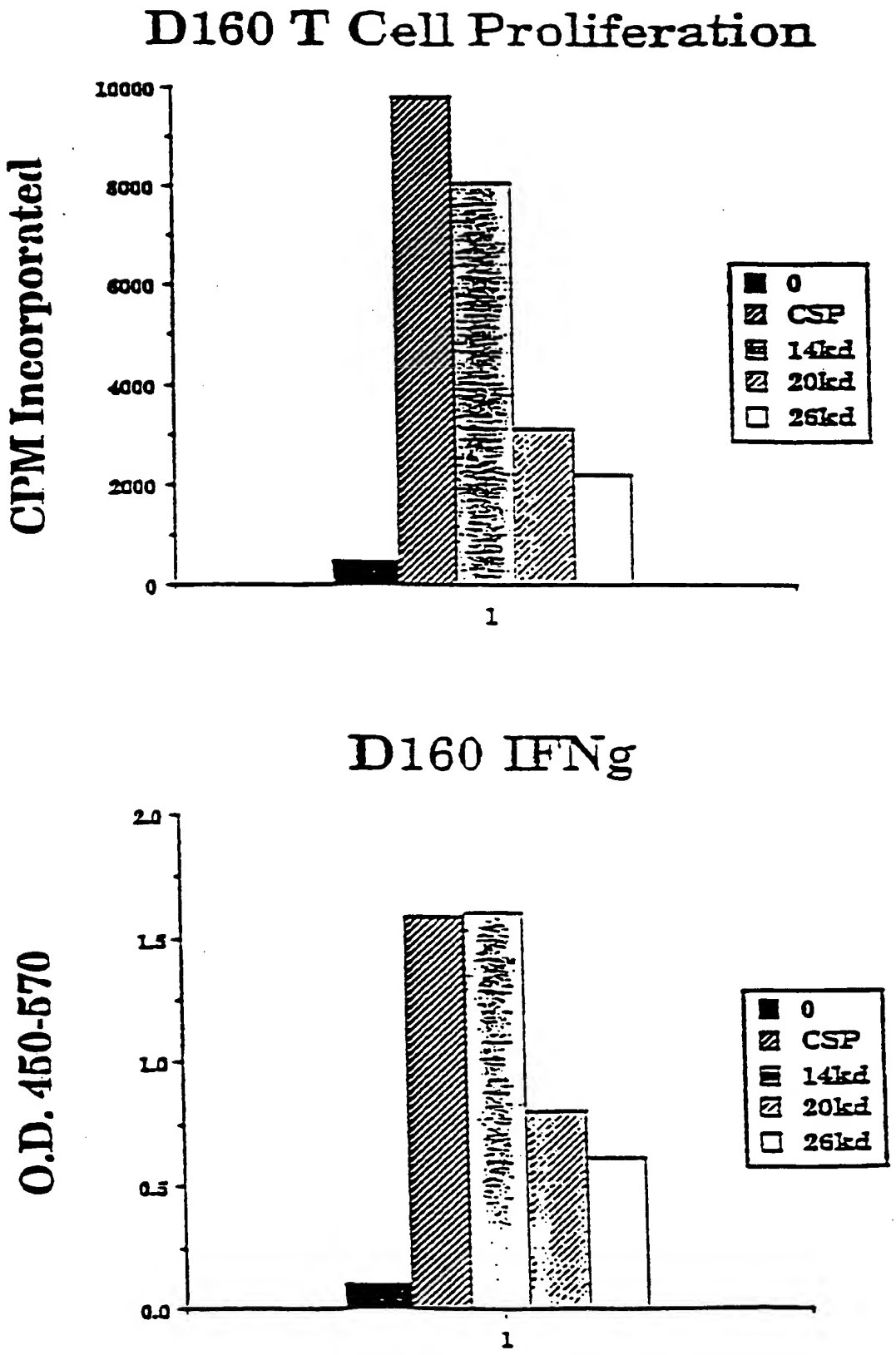

D160 T Cell Proliferation
CPM Incorporated
10000
8000
6000
4000
2000
0
1
0
CSP
14kd
20kd
26kd
D160 IFNg
O.D. 450-570
2.0
1.5
1.0
0.5
0.0
1
0
CSP
14kd
20kd
26kd

FIG. 1B

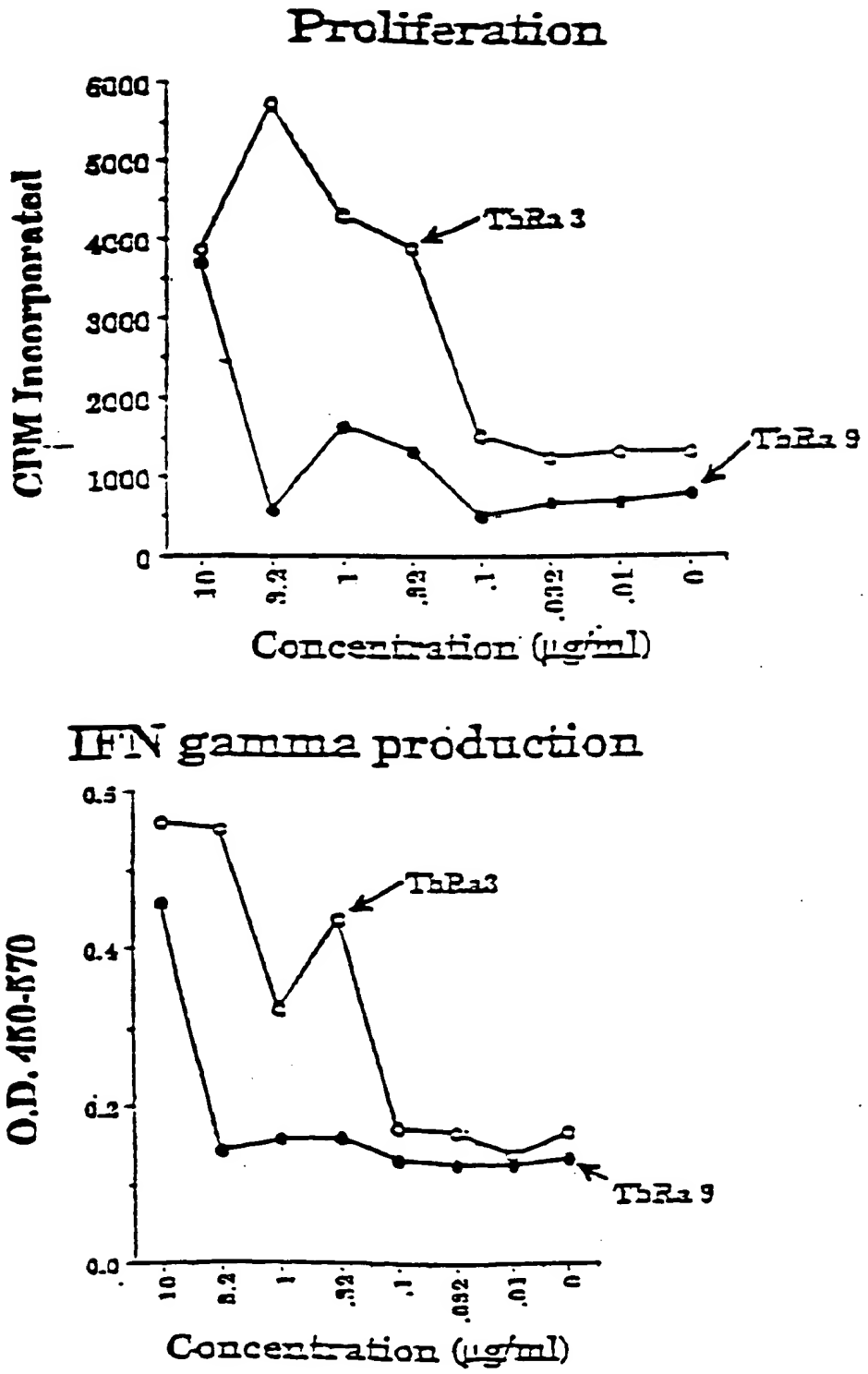
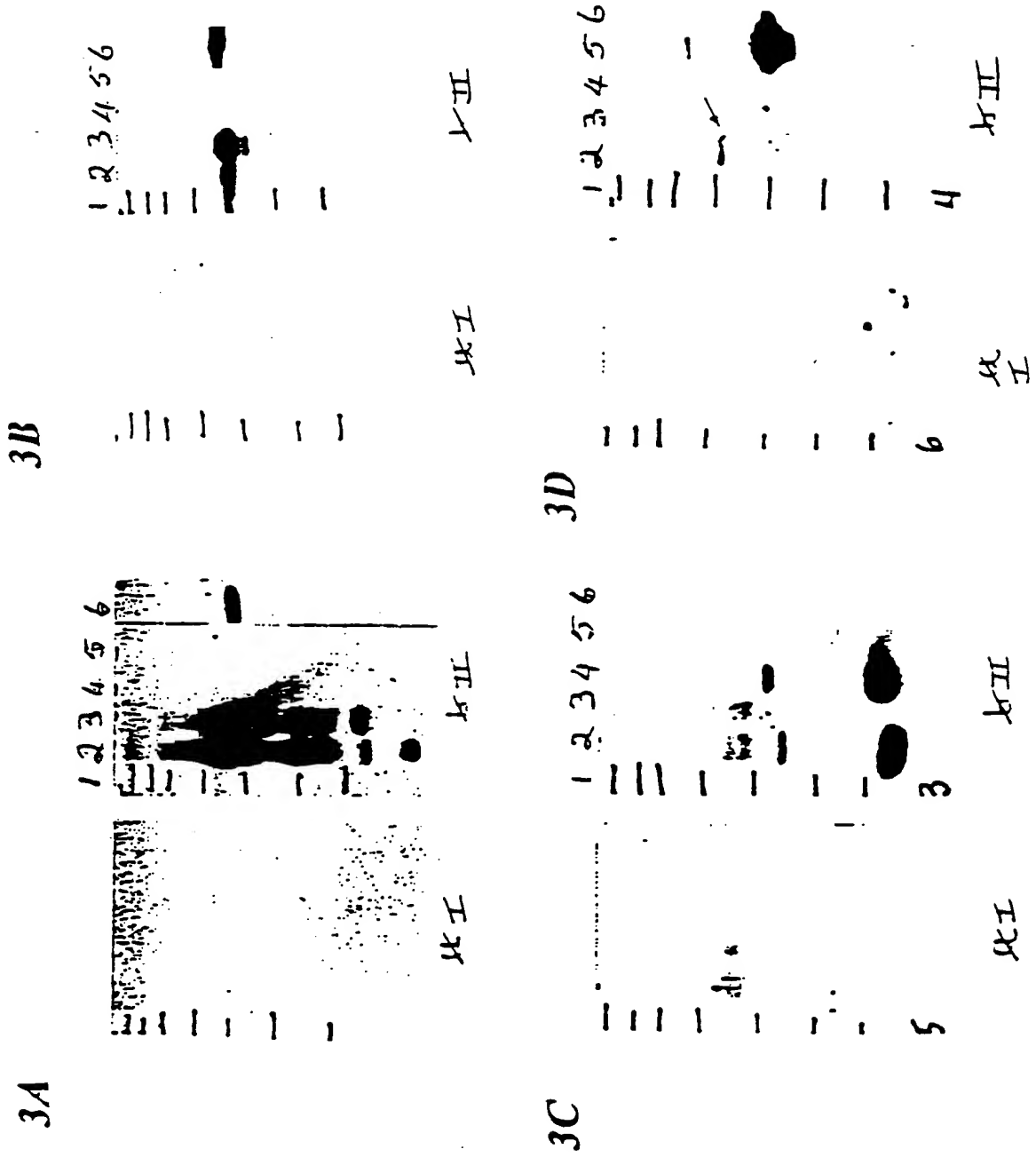
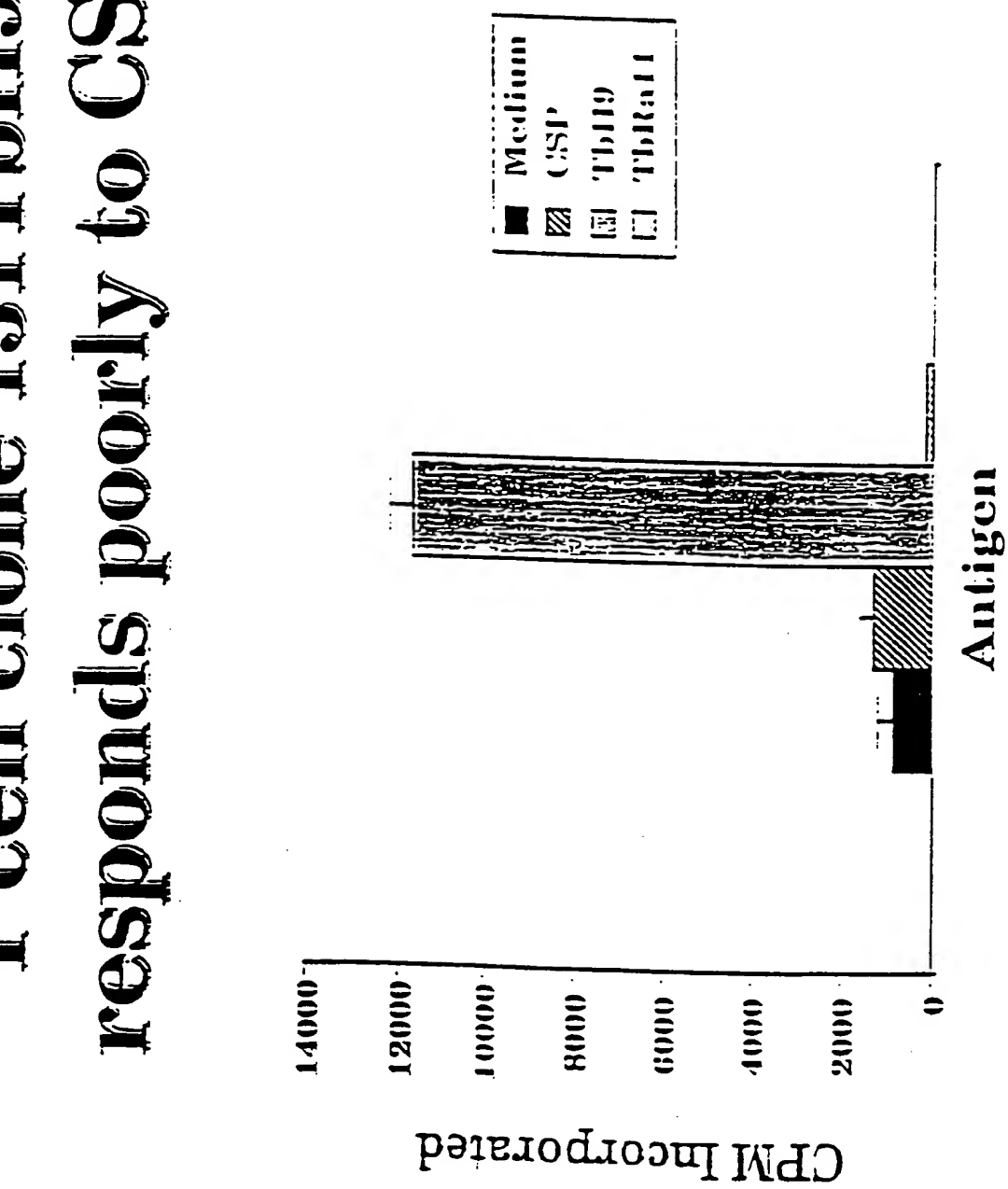

Proliferation
CPM Incorporated
6000
5000
4000
3000
2000
1000
0
TbRa 3
TbRa 9
10
3.2
1
.32
.1
.032
.01
0
Concentration (µg/ml)
IFN gamma production
O.D. 450-570
0.6
0.4
0.2
0.0
TbRa3
TbRa 9
10
3.2
1
.32
.1
.032
.01
0
Concentration (µg/ml)

FIG. 2



FIGS. 3A-D

T cell clone 131TbH9 responds poorly to CSP



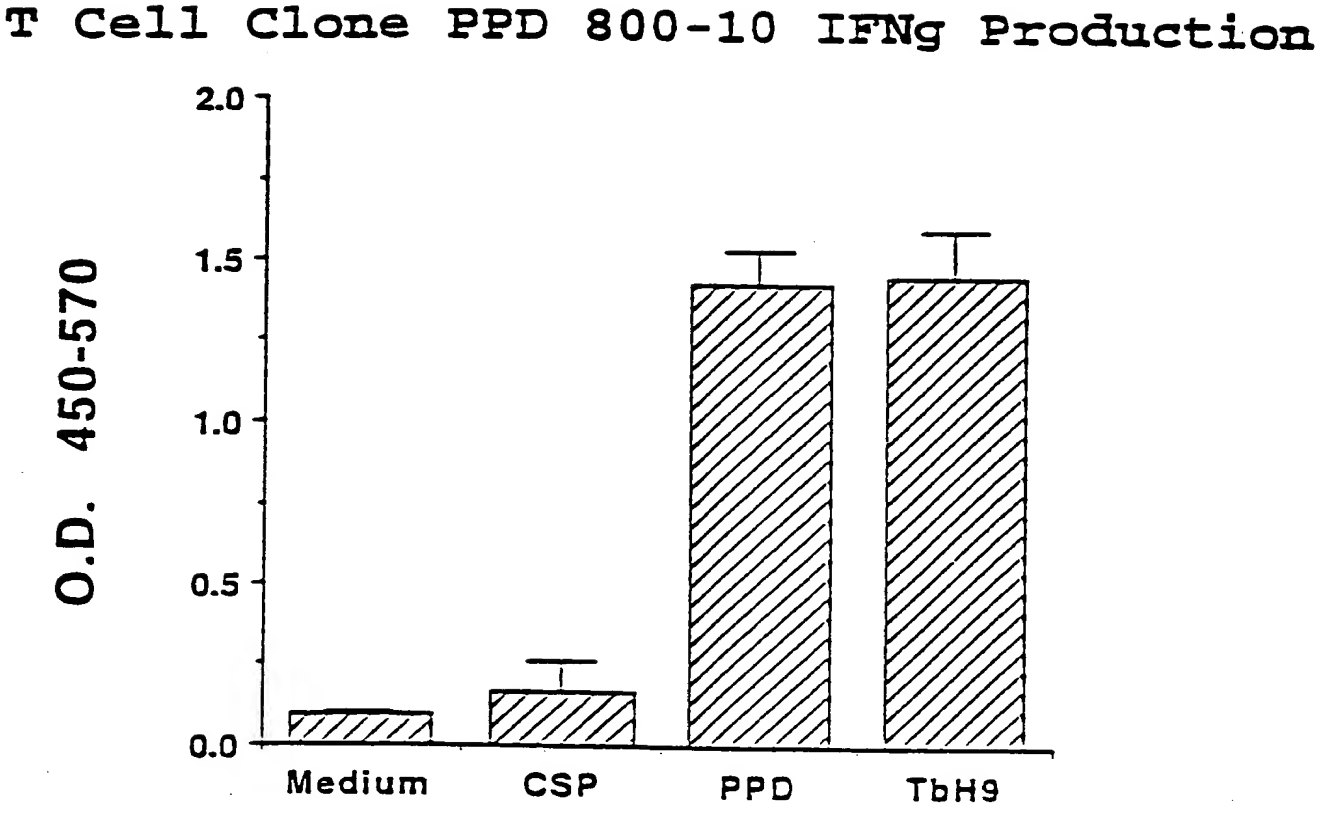
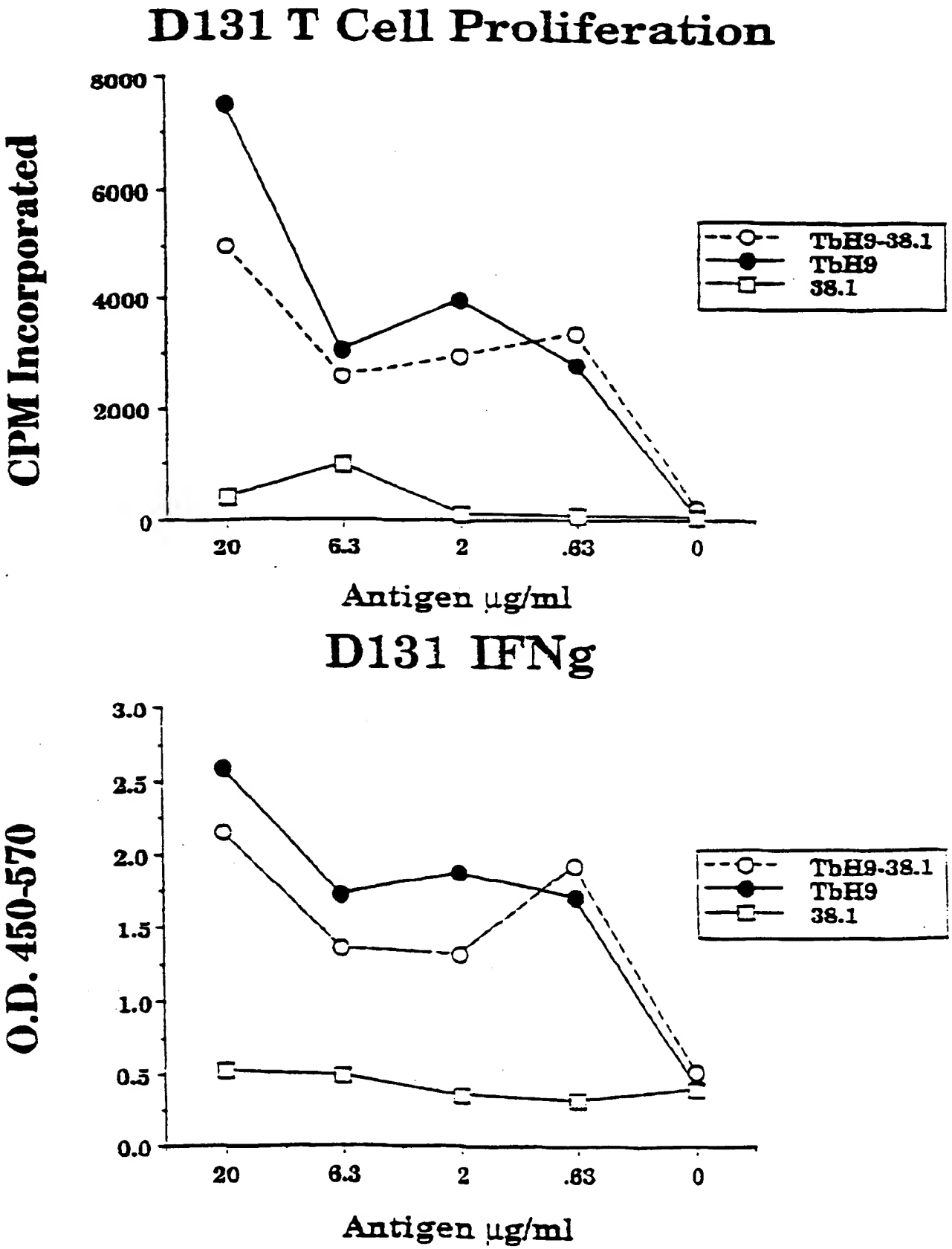
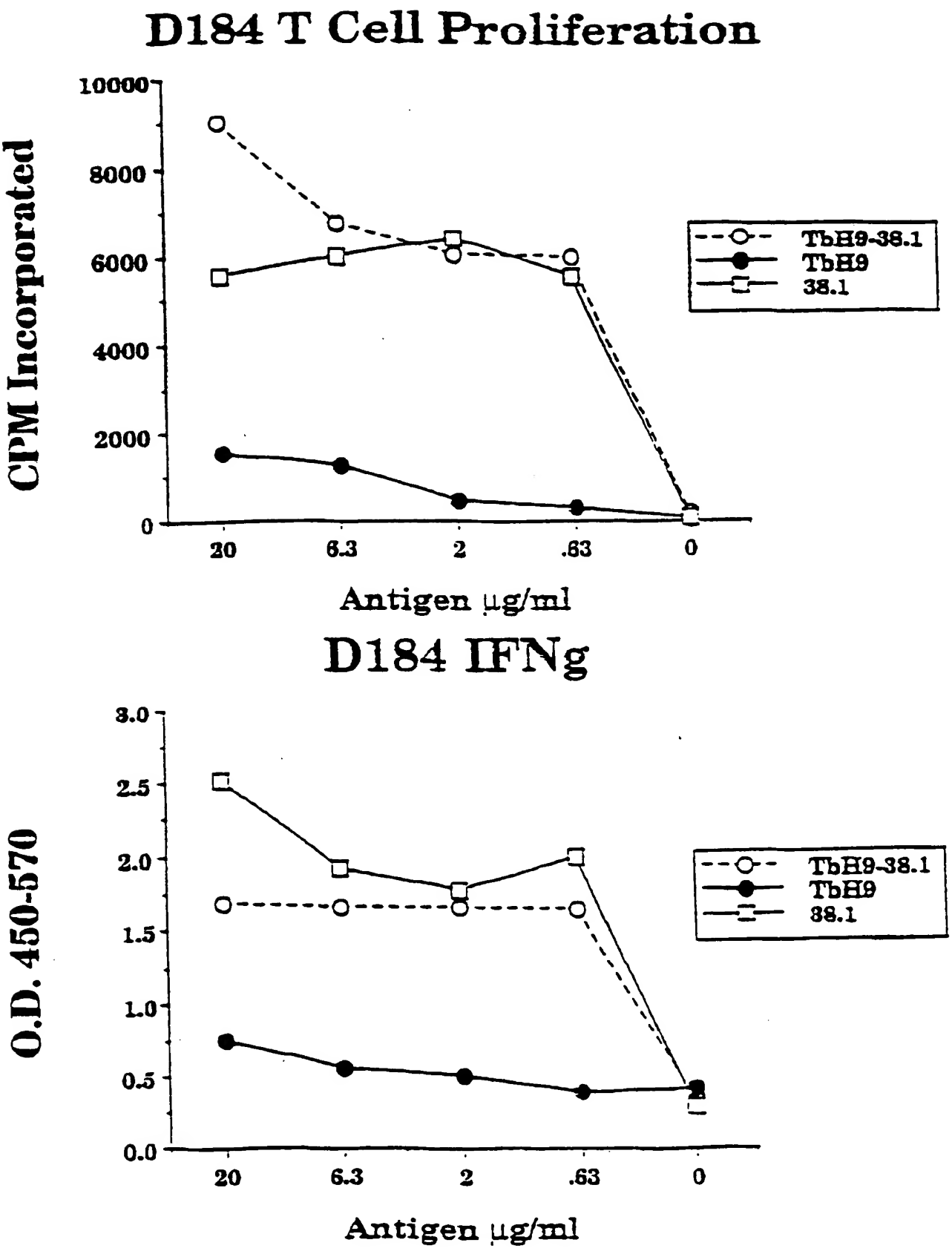

T Cell Clone PPD 800-10 IFNg Production
O.D. 450-570
2.0
1.5
1.0
0.5
0.0
Medium
CSP
PPD
TbH9

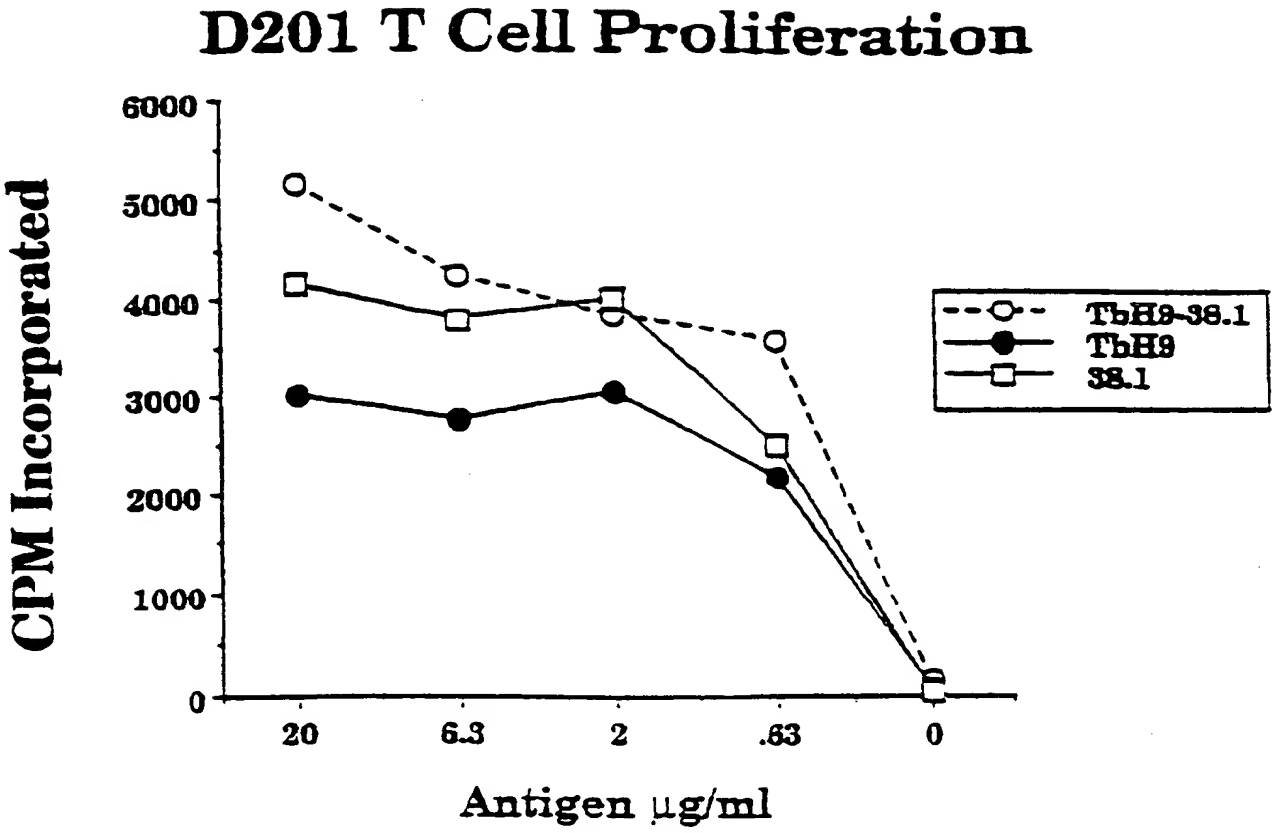
FIG. 4B

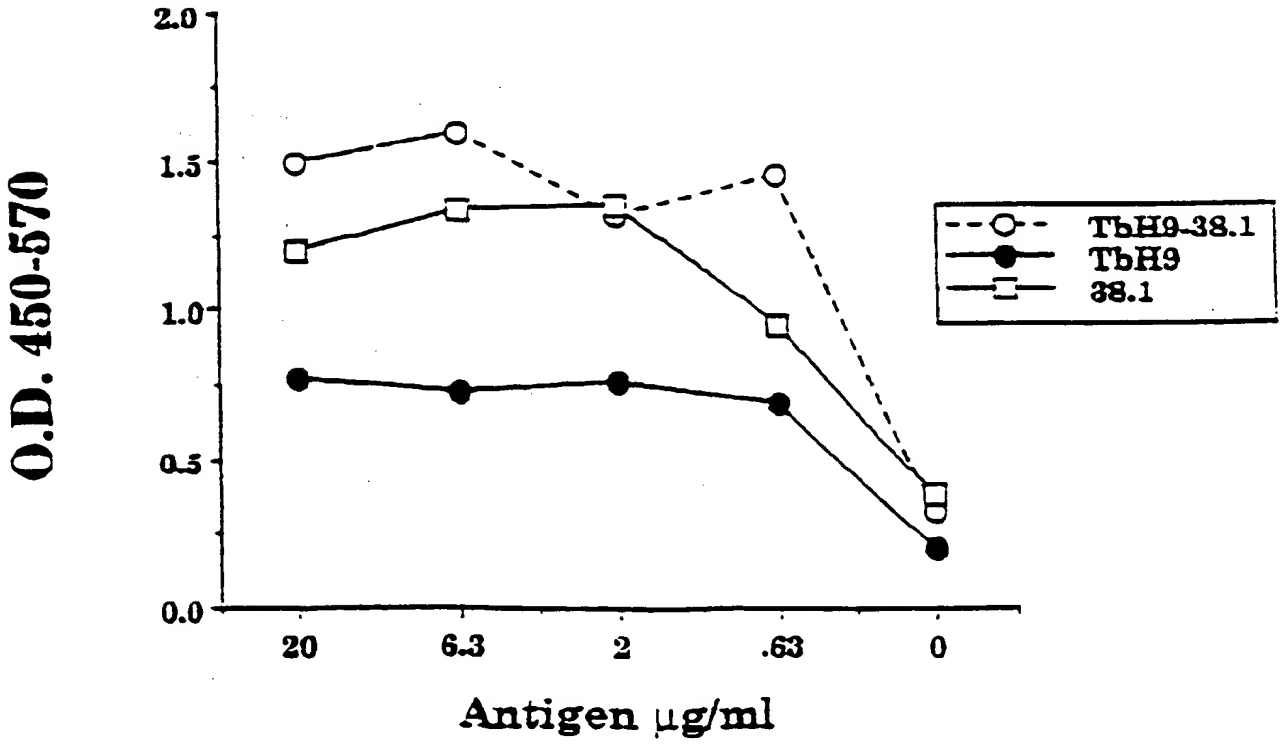

D131 T Cell Proliferation
CPM Incorporated
8000
6000
4000
2000
0
20
6.3
2
.63
0
Antigen µg/ml
TbH9-38.1
TbH9
38.1
D131 IFNg
O.D. 450-570
3.0
2.5
2.0
1.5
1.0
0.5
0.0
20
6.3
2
.63
0
Antigen µg/ml
TbH9-38.1
TbH9
38.1

FIGS. 5 A-B

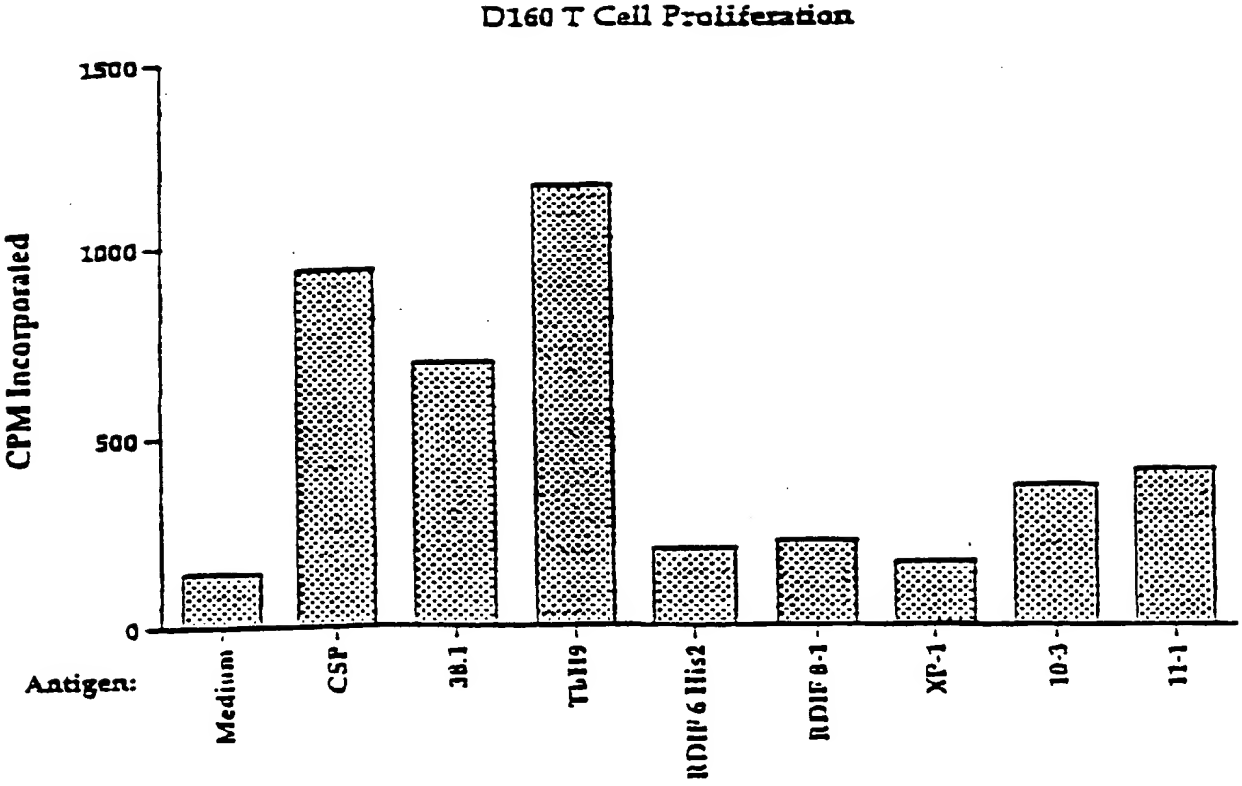

D184 T Cell Proliferation
CPM Incorporated
10000
8000
6000
4000
2000
0
20
6.3
2
.63
0
Antigen µg/ml
TbH9-38.1
TbH9
38.1
D184 IFNg
O.D. 450-570
3.0
2.5
2.0
1.5
1.0
0.5
0.0
20
6.3
2
.63
0
Antigen µg/ml
TbH9-38.1
TbH9
38.1

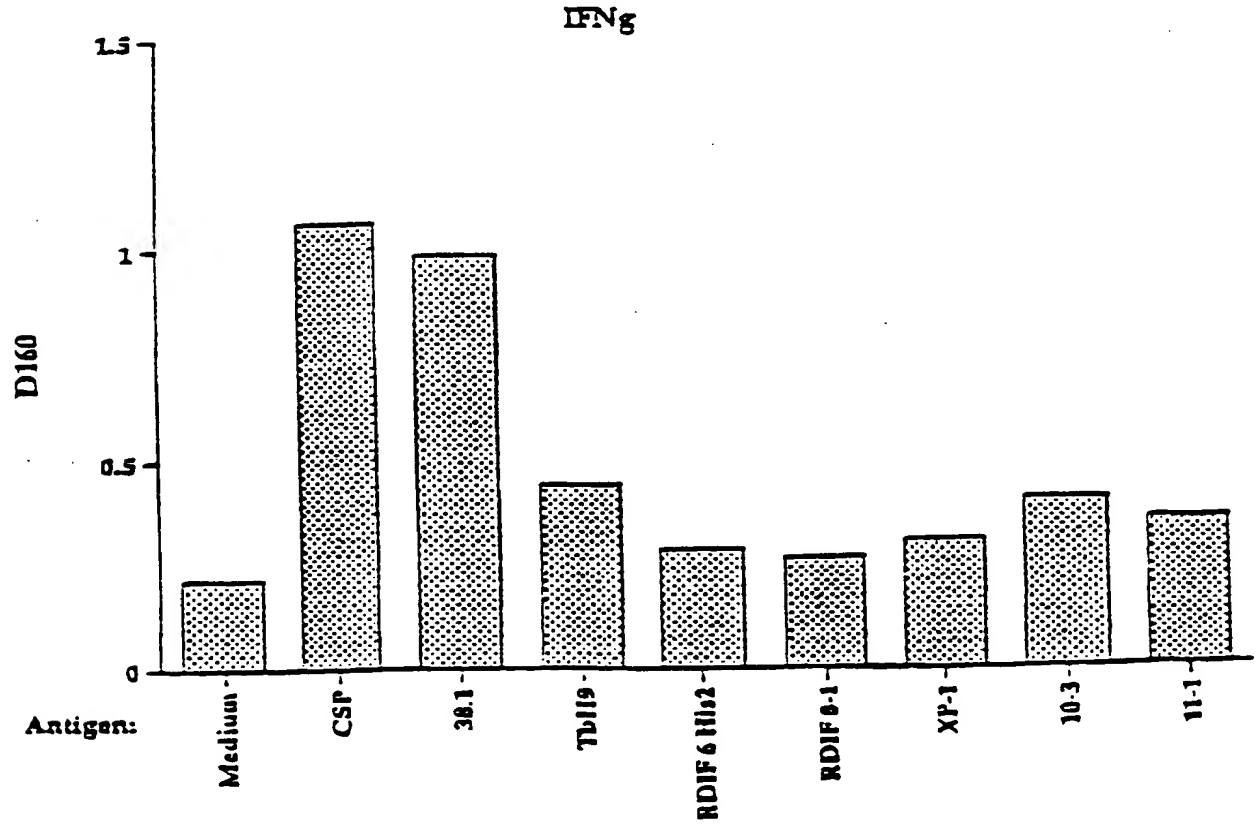
FIGS. 6 A-B


D201 T Cell Proliferation
CPM Incorporated
6000
5000
4000
3000
2000
1000
0
20
6.3
2
.63
0
Antigen µg/ml
TbH9-38.1
TbH9
38.1

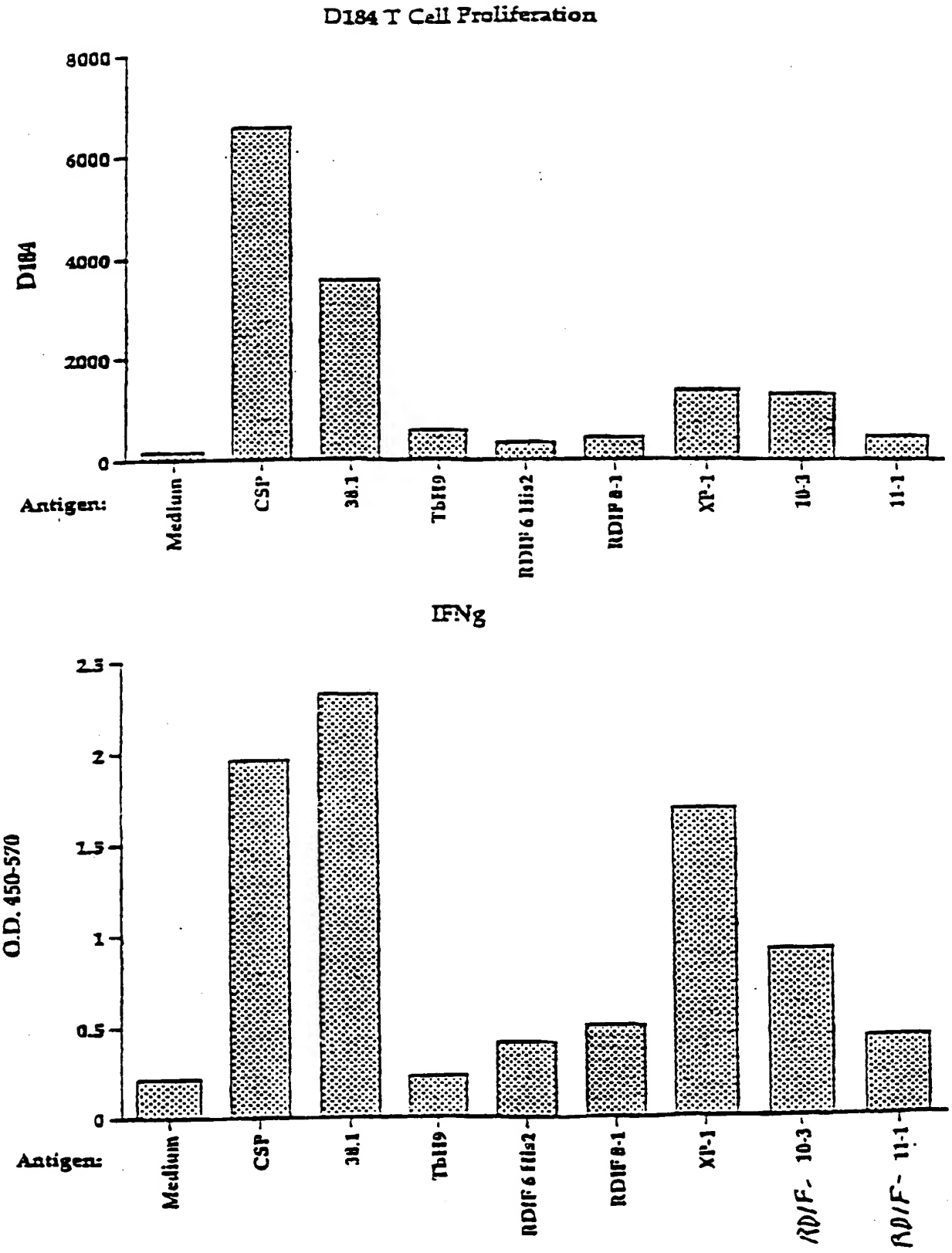

D201 IFNg
O.D. 450-570
2.0
1.5
1.0
0.5
0.0
20
6.3
2
.63
0
Antigen µg/ml
TbH9-38.1
TbH9
38.1

FIGS. 7 A-B


D160 T Cell Proliferation
CPM Incorporated
1500
1000
500
0
Antigen:
Medium
CSP
38.1
Tb119
RDIF 6 His2
RDIF 8-1
XP-1
10-3
11-1


IFNg
D160
1.5
1
0.5
0
Antigen:
Medium
CSP
38.1
Tb119
RDIF 6 His2
RDIF 8-1
XP-1
10-3
11-1

FIGS. 8A-B


D184 T Cell Proliferation
D184
8000
6000
4000
2000
0
Antigen:
Medium
CSP
38.1
Tb119
RDIF 6 His2
RDIF 8-1
XP-1
10-3
11-1
IFNg
O.D. 450-570
2.5
2
1.5
1
0.5
0
Antigen:
Medium
CSP
38.1
Tb119
RDIF 6 His2
RDIF 8-1
XP-1
RDIF- 10-3
RDIF- 11-1

FIGS. 9A-B